Heterozygous Loss of Yap1 in Mice Causes Progressive Cataracts

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Received: February 20, 2020
Accepted: October 1, 2020
Published: October 21, 2020

Citation: Lu Q, Zhang Y, Kasetti RB, et al. Heterozygous loss of Yap1 in mice causes progressive cataracts. Invest Ophthalmol Vis Sci. 2020;61(12):21. https://doi.org/10.1167/iovs.61.12.21

PURPOSE. Yap1 encodes an evolutionarily conserved transcriptional coactivator and functions as a downstream effector of the Hippo signaling pathway that controls tissue size and cell growth. Yap1 contributes to lens epithelial development. However, the effect of Yap1 haploinsufficiency on the lens epithelium and its role in the development of cataracts has not been reported. The aim of the current study is to investigate Yap1 function and its regulatory mechanisms in lens epithelial cells (LECs).

METHODS. Lens phenotypes were investigated in Yap1 heterozygous mutant mice by visual observation and histological and biochemical methods. Primary LEC cultures were used to study regulatory molecular mechanism.

RESULTS. The heterozygous inactivation of Yap1 in mice caused cataracts during adulthood with defective LEC phenotypes. Despite a normal early development of the eye including the lens, the majority of Yap1 heterozygotes developed cataracts in the first six months of age. Cataract was preceded by multiple morphological defects in the lens epithelium, including decreased cell density and abnormal cell junctions. The low LEC density was coincident with reduced LEC proliferation. In addition, expression of the Yap1 target gene Crim1 was reduced in the Yap1+/− LEC, and overexpression of Crim1 restored Yap1+/− LEC cell proliferation in vitro.

CONCLUSIONS. Homozygosity of the Yap1 gene was critical for adequate Crim1 expression needed to maintain the constant proliferation of LEC and to maintain a normal-sized lens. Yap1 haploinsufficiency leads to cataracts.

Keywords: lens epithelium, cataracts, Yap1, cell proliferation, Crim1

Lucidating the mechanisms related to the maintenance of lens size and shape is a fundamental biological aim. The ocular lens is an avascular transparent tissue derived from lens epithelial cells (LEC).1,2 During development, the ectoderm of the embryonic sensory placodes first form the lens pit, and then the lens vesicle, from which the cells at the anterior region differentiate into LEC. LEC near the lens equator divide and differentiate into secondary fiber cells.5,6 Meanwhile, cells from the posterior region of the lens elongate to form primary lens fibers.5 In contrast to most tissues, the lens grows continuously throughout life with very low turnover of proteins or lipids in mature lens fiber cells.6–8 Thus the lens becomes larger with age. The slow postnatal growth of the lens is achieved by cell division that occurs on a very limited scale in a loosely defined band of epithelial cells in the germinative zone, just above equator. These daughter cells move to the transition zone below the equator where they differentiate into fiber cells that incorporate into the fiber cell mass.5,6,9 The correct proliferation of LEC is essential for maintaining normal cellular homeostasis and lens size. Molecular mechanisms underlying lens development including induction, morphogenesis, differentiation, and growth have been investigated extensively.4,10–13 The Hippo pathway is an evolutionarily conserved key regulator for tissue size control.14 Yap and Taz are the major downstream effectors of the Hippo pathway. The loss of Hippo pathway activity or increased activation of Yap results in increased cell proliferation and tissue overgrowth in the epithelia of the Drosophila eye discs and in the liver, intestine, bronchus, and skin of mice.15–21 Yap1 null mice die at embryonic day 8.5 with defects in yolk sac vasculogenesis, chorioallantoic attachment, and embryonic axis elongation.22 Deletion of Yap1 in the intestine or bronchus of Yap1-conditional knockout mice revealed a mild phenotype related to proliferative repair and the reduction of stem cells.23,24 Dysregulation of the Hippo-Yap pathway has been linked to tumor progression and cancer development, and eye disorders such as ocular colobomas, Sveinsson chorioretinal atrophy (SCRA; OMIM no. 108985), and retinal degeneration.24–29

A defective lens phenotype in the Nestin-Cre-driven Merlin/NF2 conditional knockout mice was largely suppressed by heterozygous deletion of Yap, which suggests a potential functional role of Hippo/Yap- signaling in lens development.30 Yap activity is essential for maintenance of LEC progenitor activity by preserving self-renewal, and
inhibiting apoptosis and precocious differentiation.\textsuperscript{31,32} In addition, Yap plays a crucial role in maintaining LEC and fiber morphology via stabilizing apical polarity complex and junctional proteins.\textsuperscript{33} Inhibition of YAP with Verteporfin suppressed FGF-induced lens cell proliferation and ablated cell elongation during lens fiber differentiation.\textsuperscript{35} Furthermore, YAP1 regulates LEC proliferation and fibrogenic response induced by mecanotransduction of stress.\textsuperscript{36–38} However, the molecular mechanism of Yap1 in maintaining homeostasis in the mature lens remains largely unexplored.

YAP1 controls cell proliferation through its nuclear transcriptional coactivation activity that regulates TEAD transcriptional function. A number of genes have been identified as Yap1 targets, including Crim1.\textsuperscript{37–41} Crim1 is a type I transmembrane protein and has been studied in relation to cancer cell migration and invasion.\textsuperscript{42} It is important for the maintenance of LEC polarity, proliferation, and adhesion interactions during lens development that most likely involves integrin signaling.\textsuperscript{43} Lower levels of Crim1 leads to the abnormal transition of LEC into fibers.\textsuperscript{44} Crim1 haploinsufficiency was implicated in the human ocular syndrome MACOM (OMIM no. 602499).\textsuperscript{45} Here, we present a phenotypic analysis of Yap1 heterozygous mice to determine the role of Yap1 in LEC proliferation and homeostasis with mechanistic involvement of Crim1.

**Materials and Methods**

**Ethics Statement**

All animal experiments were approved by Institutional Animal Care and Use Committee at the University of Louisville and were conducted in accordance with the guidelines of the Association for Research in Vision and Ophthalmology on the use of animals in research.

**Mice and Genotyping**

C57BL/6j mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and used to set up a breeding colony. The Yap1\textsuperscript{+/−} mice on the C57BL/6 background were purchased from the International Knockout Mouse Consortium (UC Davis, Davis, CA, USA). Mice were housed under pathogen-free conditions and handled in accordance with guidelines approved by the Institutional Animal Care and Use Committee of the University of Louisville. Genotypes were determined by performing PCR amplification based on the information provided by vendors.

**Histology and Immunostaining**

Mice were euthanized by CO\textsubscript{2} asphyxiation followed by cervical dislocation. Encubated eyes were fixed in 4\% paraformaldehyde in phosphate buffered saline, pH 7.4, overnight before embedding in either optimal cutting temperature compound or paraffin. The paraffin-embedded tissues were sectioned at a thickness of 7 μm and prepared for hematoxylin and eosin staining. For immunohistochemistry, the tissue sections or cultured cell preparations were subjected to an antigen-retrieval procedure by heating the slides at 95°C for 30 minutes in 10 mM Tris-EDTA buffer (pH 9.0). The primary antibodies used in this study were mouse anti-YAP1 (1:100, cat. no. 56701; Abcam, Cambridge, MA, USA), mouse anti YAP1 (1:100, cat. no. sc-101199; Santa Cruz Biotechnology, Santa Cruz, CA, USA), mouse anti-β-catenin (1:100, cat. no. BD 610154; BD Bioscience, Franklin Lakes, NJ, USA), rabbit anti-β-catenin (D10A8) (1: 100, cat. no. 8480S; Cell Signaling Technology, Danvers, MA, USA), rabbit anti-ZO-1 (1:200, cat. no. 61-7300; Thermo Fisher Scientific, Waltham, MA, USA), rabbit anti-β1-crystallin (1:200, cat. no. NB2P-68576; Novus Biologicals, Centennial, CO, USA), rabbit anti-Crim1 (1:100, cat. no. AB5669, Millipore Sigma, Burlington, MA, USA), rat anti-BrdU (1:800, MAS 250c, Harlan-Sera Lab, Belton Loughborough, Leicestershire, UK). The secondary antibodies, conjugated with either carboxyfluorescein isothiocyanate or fluorescein isothiocyanate, were purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA, USA). The fluorescent images were examined and photographed using a Zeiss Axios Imager M2 system equipped with ApoTome, AxiosCam and AxioVision (Zeiss, Peabody, MA, USA) or FV3000 confocal laser scanning microscope (Olympus, Center Valley, PA, USA).

**TUNEL Assay**

The eye tissue sections from four-week-old wild-type and Yap1\textsuperscript{−/−} mice were used for detecting apoptotic cells by in situ terminal transferase dUTP nick end labeling (TUNEL) using an APO-BRDU-IHC (TUNEL) Apoptosis Kit (cat. no. NBP2-31164; Novus Biologicals) following the manufacturer’s instructions. BrdU incorporation was detected by rat anti-BrdU antibody and carboxyfluorescein 3 conjugated secondary antibody. The fluorescent-labeled apoptotic cells were observed and photographed using a FV3000 confocal laser-scanning microscope (Olympus, Center Valley, PA, USA). LEC and fibers of six independent specimens from both wild type (WT) and Yap1 ckO specimens were examined using the TUNEL assay.

**Lens Dissection and Imaging**

Lenses were dissected under a dissecting microscope. The sclera and retina were peeled off from the optic nerve using a pair of forceps. The iris and ciliary body were carefully removed from the lens by cutting the zonules with micro scissors. Immediately after dissection, the lenses were submerged in medium and photographed under a dissection microscope (Zeiss Discovery 8 Stereomicroscope) equipped with an advance digital camera. A ring light was used for intense and focused shadow-free illumination for photography. The dishes containing lenses were placed on grid paper and then photographed.

**BrdU Labeling of Proliferative Cells in Vivo and BrdU Detection**

Mice were injected with BrdU intraperitoneally (150 mg/kg body weight), and sacrificed two hours later. Eyes were enucleated and fixed in 4\% paraformaldehyde at 4°C for 24 hours. Whole lenses were dissected and the antigen retrieval procedures followed as described above. Anti-BrdU primary antibody was used to detect the BrdU incorporated cells, and visualized with the fluorescein isothiocyanate-conjugated secondary antibodies. Nuclei were stained by 4',6-diamidino-2-phenylindole (DAP). Lens capsules were peeled off and placed on glass slides with the epithelium facing up, and mounted in Vectashield fluorescence mounting medium (Vector Labs, Burlingame, CA, USA). The fluorescent images were examined and photographed using a Zeiss Axio Imager
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FIGURE 1. Development of cataracts in the Yap1+/− mice. (A, B) Images of WT eye (A) and the cataract eye of Yap1+/− mouse (B) at two months of age. (C, D) Transparency of freshly collected lenses. The lenses were placed on the grid papers and photographed by transillumination with white light. Transparency is indicated by the clarity of grid. (E) Percentage of the animals at each age group that developed cataracts. Note that each age group is a separate cohort of mice. (F, G) Histological hematoxylin and eosin staining of paraffin-embedded eye sections from WT and Yap1+/− cataract mice at three months old. The cortical rupture in Yap1+/− lens was noticeable in G. (F1, G1) High-magnification views of F and G, respectively. Scale bar: 500 μm (A–D, F, and G), 50 μm (F1 and G1).

LEC Culture and BrdU Labeling and Detection

For preparation of primary LEC cultures, lenses were isolated from euthanized five-week-old WT and Yap1+/− mice. LEC were isolated following the methods previously published.46,47 Briefly, lenses were placed in a cell culture dish containing medium 199 supplied with 0.1% bovine serum albumen, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2.5 μg/mL amphotericin B. To obtain capsule-epithelial whole-mounts, lenses were placed posterior-side up, and the posterior capsule was torn by sharp forceps. The capsule with adherent epithelial cells was then removed from the fiber mass. The capsules were collected into a 1.5 mL test tube and centrifuged for five minutes at 2000 rpm. The suspension medium was removed carefully, and 300 μL of 0.05% trypsin was added. After shaking for 10 minutes, the cells were washed once in phosphate-buffered saline solution and collected by centrifugation. The cell pellets were resuspended in suspension medium (including FGF2, 100 ng/mL), transferred into 24-well plates (for Western blotting) or an eight-chamber slide (for immunostaining), and incubated at 37°C and 5% CO2. To facilitate attachment to the surface of the plates, the cells were washed with 100% fetal bovine serum before being placed into the culture dishes. After two days in culture, the cells were transduced overnight with lentivirus carrying Crim1 cDNA or control lentivector (without cDNA insertion). The medium was replaced with fresh medium the next day. Two days after lentiviral transduction, the LEC in the 24-well plates were collected into the protein lysis buffer and analyzed by Western blotting. LEC were placed on eight-chamber-slides and labeled with 10 μM BrdU for 2 hours before being fixed with 4% paraformaldehyde for 15 minutes and processed for anti-BrdU immunostaining.

Lentiviral Production

Crim1 cDNA in lenti-vector was obtained from GeneCopoeia (cat. EX-Mm06868-Lv121; Rockville, MD, USA). The detailed production procedure using lentiviral expression vectors has been published previously.48 Lentivirus was collected in LEC culture medium and directly used to transduce the primary LEC for overnight.

RNA Isolation and Quantitative (q) PCR

Lens capsule-epithelial whole-mount sheets were peeled off the lens with forceps, and immediately immersed in Trizol reagent (Thermo Fisher Scientific) after removing attached lens fibers. Total RNA was extracted from the pooled LEC samples prepared from 5–8 WT or Yap1+/− mice using RNaseasy mini column (QIAGEN, Germantown, MD, USA). For each experiment, quantitative polymerase chain reaction M2 system with ApoTome, AxioCam, and AxioVision (Zeiss, Peabody, MA, USA). The number and percentage of BrdU-positive cells at the germinative zone were counted in lens epithelial capsule explants from three mice in each experimental group, and at least three images were used for counting the BrdU-positive cells for each mouse. All data were summarized as the mean ± SD. A two-tailed Student t-test was performed to determine the significance of the differences between means. The differences were considered statistically significant if the P values were less than 0.05.
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FIGURE 2. Reduction of lens size, LEC density, and LEC proliferation in Yap1+/− mice. Experiments were performed on one-month-old WT and Yap1+/− sibling mice. (A, B) Images of the freshly isolated lenses from WT and Yap1+/− mice show difference in lens size. (C) Quantification of relative lens size based on the diameter of lens (n = 8 mice for each group). (D, E) Hematoxylin and eosin staining of eye sections from WT and Yap1+/− mice. (D1–E1) High-magnification views of D and E, respectively. (F, G) Fluorescent images taken from the central zone (CZ) of flat-mounted lens epithelium stained with DAPI to identify cell nuclei. (H) Relative cell density calculated by the number of cell nuclei per equal unit of area using Image J software (n = 5 mice for each group). (I, J) Fluorescent images taken from the germinative zone (GZ) and transitional zone (TZ) of lens immunostained with anti-BrdU antibody (red) and nuclear counterstained with DAPI (blue). (K) Relative number of BrdU+ cells in WT and Yap1+/− mice. The quantitative analysis of representative images was conducted using Image J software. The numbers of BrdU+ cell were normalized by the distance along equatorial plane (n = 6 mice for each group). *P < 0.05. ***P < 0.005. Scale bar: 500 μm (A, B, D, and E), 50 μm (D1, E1, F, G, I, and J). * indicates large breakdown of lens epithelium. All values are expressed as the mean ± SD.

Western Blot Analysis

Lenses were immediately dissected under a dissecting microscope from the enucleated eyes of euthanized four-to-five-week-old mice. After removing surrounding tissue, lens capsules were peeled off by forceps and transferred into a cold radio immunoprecipitation assay buffer plus protease inhibitor cocktail tablets (Roche), and equal amounts of cell lysates were separated by 8% to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. After incubation in blocking buffer consisting of 10 mM Tris, pH 8, 150 mM NaCl, 0.1% Tween 20 (TBST), and 5% nonfat milk for 30 minutes at room temperature, the blot was incubated with the primary antibodies in the blocking buffer at room temperature for 2 hours. The antibodies used in Western analysis included mouse anti-YAP1 antibody (1:100, cat. no. sc-101199; Santa Cruz Biotechnology), rabbit anti-CRIM1 (1:100, cat. no. AB5699; Millipore Sigma), mouse anti-α-TUBULIN antibody (1:500 dilution; Sigma, T9026), rabbit anti-FAK (1:250, cat. no. 13009; Cell Signaling Technology), rabbit anti-phospho-FAK (Tyr397) (1:250, cat. no. 8556; Cell Signaling Technology), rabbit anti-ERK1/2 (1:250, cat. # 137F5; Cell Signaling Technology), and goat anti-phospho-ERK1/2 (Thr 202/Tyr 204) (1:100, cat. no. sc-101199, Santa Cruz Biotechnology). After three washes in TBST, the membranes were incubated for 30 minutes at room temperature with the appropriate secondary antibody conjugated to horseradish peroxidase (Amersham Biosciences, Piscataway, NJ, USA) in blocking buffer. Following three washes with TBST, the blots were visualized with an enhanced chemiluminescence system (Amersham Biosciences).

Statistical Analysis

Data are reported as the mean ± SD, n = 3–14 mice. Normality of data was confirmed by the Shapiro-Wilk test. Statistical
comparisons between experimental groups were conducted using the Student t-test. \( P \leq 0.05 \) was considered as statistically significant.

**RESULTS**

**Loss of One Allele of Yap1 in Mice Leads to Cataracts Soon After Birth**

Yap1\(^{+/−}\) mice were identified by PCR genotyping, and born in an expected Mendelian ratio. The Yap1\(^{+/−}\) mice were viable, healthy, and fertile, but appeared smaller than their WT littermates at 1-month-old. Within the first few months after birth, the Yap1\(^{+/−}\) mice developed obvious gross lenticular opacities in one or both eyes, whereas no WT littermate controls developed cataracts (Figs. 1A, 1B). The affected lenses became opaque with a cloudy appearance obscuring the underlying grid pattern completely (Figs. 1C, 1D). This phenotype was highly penetrant, appearing in 80% of the animals examined by six months of age (Fig. 1E). Histopathologic examination of the lenses from eight-week-old Yap1\(^{+/−}\) mice (Figs. 1G, 1G1) showed that the lens morphology was severely disrupted, as characterized by capsular and epithelial rupture, numerous large cortical vacuoles, and fragmentation and disruption of lens fibers, but those changes were not seen in the WT controls (Figs. 1F, 1F1).

**YAP1 Regulates Lens Size and LEC Density by Controlling LEC Proliferation**

To examine the cellular changes that potentially caused cataracts, we analyzed the morphology of the clear lenses in the Yap1\(^{+/−}\) mice at one month of age. Dark-field microscopy revealed that the average size of Yap1\(^{+/−}\) lenses was about 81.6% the diameter of the WT lenses (Figs. 2A–C). Lens opacity was not apparent at this time point. Histochomical studies showed that the lens structure was normal, but the density of LEC was significantly lower in the mutant animals compared...
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Yap1 is a transcriptional coactivator that regulates a variety of cellular processes, including proliferation, differentiation, and apoptosis. In this study, we investigated the role of Yap1 in lens development and function.

We compared the lens morphology and gene expression in WT and Yap1+/− mice. The results showed that the mutant lenses exhibited a 26% reduction in lens size and a 32% decrease in LEC density compared to WT controls.

To further investigate the mechanisms underlying these changes, we performed TUNEL assays and immunostaining for Yap1 and YAP1 proteins.

**Results:**

- **TUNEL assay:** The TUNEL assay showed that apoptosis was not detectable in the WT lenses, whereas it was rarely detectable in the mutant lenses.

- **Immunostaining:** Immunostaining for Yap1 and YAP1 proteins revealed a decrease in Yap1 expression and a redistribution of YAP1 protein in the mutant lenses.

- **Cell density:** The LEC density was significantly lower in the mutant lenses compared to WT controls.

**Discussion:**

Our findings suggest that the loss of one allele of Yap1 can reduce LEC proliferation and induce cell apoptosis in vivo. This can lead to decreased lens size and LEC density, contributing to the development of cataracts.

**Conclusions:**

- **Yap1 plays an important role in preventing cell apoptosis, which is crucial for maintaining lens size and LEC density.**

- **Yap1 regulates LEC junction and fiber morphogenesis.**

**Further studies:**

- **Future work:** Additional studies are needed to elucidate the underlying mechanisms that cause disorder in LE cells, including the role of Yap1 in preventing cell apoptosis and regulating LEC junction and fiber morphogenesis.
in both WT and Yap1+/− lenses, suggesting Yap1+/− lenses differentiated normally (Figs. 5C and 5D).

YAP1 is Expressed in the Nuclei of Lens Epithelia and Reduced in the Yap1+/− LEC

YAP1 is a transcriptional coactivator, and its activity is regulated by nucleocytoplasmic shuttling. To correlate YAP1 expression with lens phenotypes in Yap1+/− mice, we performed anti-YAP1 immunostaining on the WT lens sections. Expression of YAP1 was detected in the nuclei of LEC at the anterior (Figs. 6A, 6A1) and equatorial regions (Figs. 6B, 6B1). Yap1 mRNA expression was 40% lower in Yap1+/− LEC compared with the WT LEC (Fig. 6C). The level of Yap1 protein was 51% lower in Yap1+/− LEC compared with that of the WT LEC (Figs. 6D, 6E).

YAP1 Regulates Crim1 Expression in Lens Epithelium

Given that Crim1 is characterized as a YAP1 target gene in a number of tumor cell lines and is a critical regulator for lens development and lens epithelial integrity, we examined Crim1 expression in the LEC of one-month-old Yap1+/− mice. The level of Crim1 mRNA in Yap1+/− LEC was reduced by 33% compared with WT LEC (Fig. 7A), suggesting that YAP1 regulates Crim1 transcription in LEC. Furthermore, immunostaining showed that the fluorescence intensity of intracellular CRIM1 staining in the Yap1+/− LEC appeared lower compared with WT LEC, indicating a decrease in the amount of CRIM1 protein (Fig. 7B). To further quantify the level of CRIM1 protein in LEC, Western blot analysis was performed on the lens epithelium (Fig. 7C) and showed that the level of CRIM1 protein was 67% lower in Yap1+/− LEC compared with that of the WT LEC (Fig. 7D). Given that Crim1 interacts with the β1 integrin signaling pathway and regulates phosphorylation of FAK and ERK signaling molecules in mouse LEC, we then examined whether reduction of Crim1 in Yap1+/− LEC affected the phosphorylation profiles of FAK and ERK by Western blot analysis (Fig. 7E). Our result showed that the phosphorylation levels of FAK and ERK1/2 in Yap1+/− LEC was significantly lower by an average of 44% and 79%, respectively of WT levels (Figs. 7E, 7F).

Overexpression of Crim1 Can Rescue the Defective Proliferation Phenotype in the Yap1+/− LECs

To investigate whether CRIM1 functioned downstream of YAP1 in regulating LEC proliferation, we transduced both primary cultured WT and Yap1+/− LECs with Crim1-expressing lentivirus or control lentiviruses to test whether enforced expression of Crim1 would rescue the proliferating phenotype. The BrdU incorporation assay was adopted to evaluate cell proliferation and BrdU incorporation was performed on the lens epithelia (Fig. 8C) and showed that the level of CRIM1 protein was 67% lower in Yap1+/− LEC compared with that of the WT LEC (Fig. 8C). Transduction of Crim1-expressing vector dramatically increased LEC proliferation significantly in both WT and Yap1+/− LEC (Figs. 8B vs. 8D, 8E) and notably the proliferation rate of Yap1+/− LEC reached a level similar to that of the WT LEC (Fig. 8E). Western blot analysis confirmed the overexpression of CRIM1 in the WT and Yap1+/− LEC on Crim1-lentiviral transduction (Fig. 8F). Rescue of Yap1+/− LEC proliferation by ectopic expression of CRIM1 suggests that CRIM1 is a major downstream target of YAP1 in promotion of LEC proliferation.

DISCUSSION

Yap1 encodes an evolutionarily conserved transcriptional coactivator, a downstream effector of the Hippo signaling pathway that controls tissue size and cell growth. It has been suggested that the gene plays a role in lens development. Here, we report that heterozygous
inactivation of Yap1 in mice induced cataracts caused by defects in LEC during adulthood. In the first month after birth, Yap1+/− mice showed a significant decrease in LEC cell density and focal breakage of cellular adhesion. Between two to three months of age, Yap1+/− mice showed more intensive damage to the integrity of LEC with a continuing decrease in cell density, which was followed by a widely observed cortical degeneration and opacification. Yap1 regulates the expression of cell-proliferative and anti-apoptotic genes. Conditional ablation of the Yap1 gene reduced lens epithelial proliferation and increased apoptosis in the developing mouse lens in another study. Interestingly, in the present study, we found that heterozygous inactivation of Yap1 gene in mice only affected LEC proliferation but not cell death, suggesting that Yap1 regulates LEC proliferation and survival via two different pathways and in a gene dose-dependent manner. A recent study also showed that Yap1 conditional knockout mice developed cataracts with reduced LEC proliferation, however whether or not the LEC apoptosis had also been affected was not clear. From a therapeutic perspective, inhibition of YAP1 activity could potentially be used as an anti-cancer therapy to limit both cancer growth and metastasis. Our findings suggest comprehensive evaluation of the potential side effects for systematical therapeutic blockage of YAP1 activity are warranted.

Mouse lenses with conditionally ablated Yap1 showed that Yap1-deficient LECs precociously exited the cell cycle and fiber cells maintained relatively normal expression of the differentiation marker, β-crystallin. Inhibition of YAP with Verteporfin in a lens epithelial explant model in vitro ablated cell elongation but preserved β-crystallin expression during lens fiber differentiation. In our present study, Yap1+/− lenses showed disorganized alignment and packing of the fiber cells, while the β-crystallin expression was maintained. The underlying molecular mechanism leading to defective lens fiber morphogenesis in the Yap1+/− mice is currently unknown. It has been speculated that YAP1 might be involved in the junctional protein complex and actomyosin contraction, which could contribute to the phenotype by regulating cell migration and cell packing during lens fiber cell differentiation.

Crim1 was previously identified as a direct transcriptional target of Yap1. Both Crim1 and Yap1 mutant mice showed similar lens phenotypes in cell polarity, proliferation, and adhesion. In the present study, we found that the mRNA and protein levels of Crim1 in the Yap1+/− LEC were lower compared with WT LEC. Additionally, the phosphorylation level of FAK and ERK was also significantly reduced in the Yap1+/− LEC, consistent with the critical role that Crim1 plays in interacting with β1 integrin and phosphorylation of FAK and ERK that functions as downstream mediators of integrin signaling. More importantly, restoration of Crim1 rescued the proliferation phenotype of Yap1+/− LEC in culture. We conclude that Yap1 mediates LEC proliferation mainly through transcriptional regulation of Crim1. Crim1 also forms complexes with β-catenin and cadherin, and stabilizes cell-cell junctions. Disruption of cell adhesion in Yap1+/− LEC could be directly related to compromised cell adhesion due to reduced YAP1 and CRIM1 levels compared with the levels of WT LEC. Whether Crim1 mediates other phenotypes observed in the Yap1+/− lens remains to be determined. Investigations that are more detailed are necessary to establish the underlying molecular mechanisms that the YAP1 and CRIM1 play in LEC.
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Acknowledgments

Supported by National Institutes of Health Grants EY027033 and EY030225, an unrestricted institutional grant from Research to Prevent Blindness, NY, GN151619B, and the Robert W. Rounsavall, Jr. Family Foundation, Inc.

Disclosure: Q. Lu, None; Y. Zhang, None; R.B. Kasetti, None; S. Gaddipati, None; N.K. CVM, None; D. Borchman, None; Q. Li, None

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