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Long non-coding RNA MEG3 silencing protects against light-induced retinal degeneration

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ABSTRACT

Excessive light exposure leads to retinal degeneration and accelerates the progression and severity of several ocular diseases, such as age-related macular degeneration (AMD) and retinitis pigmentosa. Long non-coding RNAs (LncRNAs) have emerged as important regulators of photoreceptor development and ocular diseases. In this study, we investigated the role of lncRNA-MEG3 in light-induced retinal degeneration. MEG3 expression was significantly up-regulated after light insult in vivo and in vitro. MEG3 silencing protected against light-induced retinal degeneration in vivo and light-induced photoreceptor cell apoptosis in vitro. Mechanistically, MEG3 regulated retinal photoreceptor cell function by acting as p53 decoy. MEG3 silencing decreased caspase 3/7 activity, up-regulated anti-apoptotic protein (Bcl-2) expression, and down-regulated pro-apoptotic protein (Bax) expression. Taken together, this study provides a promising method of MEG3 silencing for treating light-induced retinal degeneration.

1. Introduction

Light exposure is a high risk environmental factor that can damage photoreceptors. Excessive light may accelerate the progression and severity of age-related macular degeneration (AMD) and some forms of retinitis pigmentosa [1]. Long-term bright light exposure would increase oxidative stress, change macular pigmentation, and decrease visual acuity [2,3]. Generally, light-induced photoreceptor loss is irreversible in retinal degenerative disease [4]. In the animal model studies, growing evidence has revealed that antioxidant, free radical scavengers, cytokines, calcium channel blockers, glucocorticoids, erythropoietin, and hypoxic preconditioning could be used to protect photoreceptor cells against apoptosis [5–7]. However, there is no effective treatment for blindness resulting from the loss of photoreceptors in humans.

Long noncoding RNAs (lncRNAs) are a class of RNA transcripts greater than 200 nucleotides [8]. They play important roles in many biological processes, such as cell proliferation, cell differentiation, cell apoptosis, transcriptional regulation, and immune response [9,10]. The mutations and dysregulations of lncRNAs are associated with several human diseases ranging from neurodegenerative diseases to cancers [11,12]. More importantly, lncRNAs have shown as key regulators in retinal cell fate specification and photoreceptor progenitor cell differentiation [13–15]. Inspired by these evidences, we speculated that lncRNAs were potential regulators of light-induced retinal degeneration.

Maternally expressed gene 3 (MEG3) is an imprinted gene belonging to the imprinted DLK1-MEG3 locus located at chromosome 14q32.3 in humans. It encodes a lncRNA and is expressed in several tissues [16]. MEG3 gene expression is lost in a great number of human tumours and tumour cell lines, suggesting that it acts as a tumour suppressor [17–19]. MEG3 has also been reported to be associated with the pathogenesis of many nervous system diseases [20,21]. Recently, MEG3 dysregulation has been reported in several ocular diseases, such as diabetes retinopathy and retinoblastoma [22,23]. However, the role of MEG3 in light-induced retinal degeneration is still unclear.

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In this study, we investigated the role of MEG3 in light-induced retinal degeneration. MEG3 expression was significantly up-regulated after light insult. MEG3 silencing protected against light-induced retinal degeneration in vivo and light-induced photoreceptor cell apoptosis in vitro. This study indicates that lncRNA- MEG3 is promising target for the treatment of light-induced retinal degeneration.

2. Materials and methods

2.1. Animal care

All procedures were performed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. They were also approved by the Animal Ethics Committee of Nanjing Medical University, China. Wild-type C57BL/6 mice (male, 2-month old) were maintained in a 12-hour light:12-hour dark cycle with free access to food and water. Mice were sacrificed by cervical dislocation under the anesthesia induced by ketamine (80 mg/kg) and xylazine (10 mg/kg). All efforts were made to minimize suffering and reduce the number of mice used.

2.2. Light-induced retinal neurodegeneration model

After dark adaptation for 24 h, C57BL/6J mice were dilated with 1% cyclopentolate hydrochloride eyedrops (Santen) for 30 min before light exposure. The mice were exposed to 8000 lux of white light for 12 h/day for 6 successive days. The light source was provided by cool white tubes. The temperature during this experiment was maintained at 25 °C ± 2 °C. After light exposure, all mice were placed in the normal light/dark cycle [24].

2.3. Electroretinography

The mice were maintained in total darkness overnight and prepared for ERG recording under dim red light. They were dilated with 1% tropicamide and 2.5% phenylephrine (Santen). ERGs were recorded in the left eyes of dark-adapted mice by placing a golden-ring electrode (Mayo) in contact with the cornea and a reference electrode (Nihon Kohden) through the tongue. A neutral electrode (Nihon Kohden) was inserted subcutaneously near the tail. The amplitude of a-wave was calculated from the baseline to the maximum a-wave peak, and the b-wave was calculated from the maximum a-wave peak to the maximum b-wave peak [25].

2.4. Retinal histology

Each eye was enucleated and immersed in a fixative solution containing 4% paraformaldehyde for 12 h at 4 °C. Paraffin-embedded sections (Thickness, 5 μm) were cut through the optic disc of each eye and then stained with hematoxylin and eosin. The damage induced by light exposure was then evaluated with six sections from each eye used for the morphometric analysis. Light microscope images were photographed, and the thickness of outer nuclear layer (ONL) from the optic disc was measured [5].

2.5. TUNEL assay

Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining was performed according to the manufacturer's protocols (In Situ Cell Death Detection Kit; Roche Applied Science) to detect light-induced retinal degeneration [26]. The eyes were enucleated, fixed in 4% paraformaldehyde for 12 h, immersed for 24 h in 25% sucrose with PBS, and then embedded in the supporting medium for frozen-tissue specimens (OCT compound; Tissue-Tek). Ten-micrometer retinal sections were cut on a cryostat at −25 °C and stored at −80 °C until staining. The reaction mixture (50 μl TUNEL) was added to each sample and incubated in a humidified atmosphere for 1 h at 37 °C. As for the negative controls, the TdT enzyme was replaced by the label solution. The sections were observed using a fluorescent microscope.

2.6. Cell culture and light-induced damage cell model

The murine photoreceptor cell line (661W) was cultured in DMEM (Gibco) containing 10% FBS (Gibco), 100 IU/mL streptomycin, and 100 IU/mL penicillin (Gibco). The cells were exposed to 2500 lux of white light for 12 h, 24 h, or 36 h to build light-induced damage cell model.

2.7. RNA pull-down assay

MEG3 was biotin labeled using the T7 RNA polymerase (Promega, Madison, WI, USA) and Biotin RNA Labeling Mix (Roche, Indianapolis, IN, USA). A total 10 μl of cell extracts were used as 1% input sample. One mg of cell extracts were incubated with biotinylated MEG3 at room temperature for 1 h. Then, 50 μl of washed Streptavidin agarose beads (Invitrogen) were added to the extracts and incubated at room temperature for 1 h. The beads were washed three times in lysis buffer, boiled in SDS buffer. The biotinylated MEG3-bound proteins were detected by western blots [20].

2.8. RNA immunoprecipitation

Cell lysates were incubated with nonspecific IgG (IgG, 3 μg) or p53 (3 μg, Abcam) for 12 h at 4 °C. Then, 30 μl of protein G-Sepharose (Sigma) was added and incubated for 3 h at 4 °C. The precipitated RNAs were extracted using the phenol and chloroform. MEG3 expression was finally detected by qRT-PCRs.

2.9. Statistical analysis

All data are expressed as mean ± SEM. The significant difference between two groups was analyzed using Student's *t*-test (unpaired, 2-tailed). The significant difference between multiple groups was analyzed using one-way ANOVA. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Light exposure leads to increased MEG3 expression

Increasing evidence suggests that long-term light insult is a high-risk factor for development and progression of retinal degeneration [1]. In light-induced retinal neurodegeneration mouse model, light exposure led to a significant increase in retinal MEG3 expression (Fig. 1A). Light exposure could lead to retinal photoreceptor loss [5]. The murine photoreceptor cells (661W) were exposed to 2500 lux of white light for 12 h, 24 h, and 36 h. We showed that light insult significantly up-regulated MEG3 expression in vitro (Fig. 1B). Long-term light exposure could also up-regulate the level of oxidative stress [27]. We also showed that oxidative stress led to a marked increase in MEG3 expression in vitro (Fig. 1C). Taken together, these results show that MEG3 expression is significantly up-regulated after light insult in vivo and in vitro.

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