Experimental Eye Research 106 (2013) 24–33

Contents lists available at SciVerse ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Expression and localization of CERKL in the mammalian retina, its response to light-stress, and relationship with *NeuroD1* gene

Nawajes A. Mandal [a,b,c,*], Julie-Thu A. Tran [a,c], Anisse Saadi [a,c], Abul K. Rahman [a,c], Tuan-Phat Huynh [a,c], William H. Klein [d], Jang-Hyeon Cho [d,1]

[a] Department of Ophthalmology, OUHSC, Dean A. McGee Eye Institute, Oklahoma City, OK 73104, United States
[b] Department of Physiology, OUHSC, Dean A. McGee Eye Institute, Oklahoma City, OK 73104, United States
[c] Dean A. McGee Eye Institute, Oklahoma City, OK 73104, United States
[d] Department of Biochemistry and Molecular Biology, The University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030, United States

ARTICLE INFO

Article history:
Received 23 May 2012
Accepted in revised form 26 October 2012
Available online 8 November 2012

Keywords:
ceramide kinase like (CERKL)
ceramide kinase (CERK)
NeuroD1
retina
photoreceptor
light-induced retinal degeneration
gene expression

ABSTRACT

Mutations in the *Ceramide kinase like* (*CERKL*) gene are associated with retinitis pigmentosa (RP26) and cone-rod dystrophy. CERKL is homologous to *Ceramide kinase* (CERK), and its function is still unknown. The purpose of this study was to test the expression and distribution of this gene and its protein in rat and in mouse tissues, in light-stressed rat retinas and in the retinas of *NeuroD1* knock-out mice to understand the role of CERKL in the retina. Expression of *Cerkl* and *Cerk* mRNA was determined by quantitative RT-PCR. Expression of the protein was determined by Western blotting with anti-CERKL antibody. Localization of the protein was determined by using immunofluorescence microscopy. With qRT-PCR, we revealed that the relative mRNA expression of *Cerkl* was the highest in the retina among all the rat tissue tested; it was >10-fold higher than in the brain. On the other hand, *Cerk* has ubiquitous expression and its relative abundance is >2 fold of *Cerkl* in the retina. *Cerkl* was expressed minimally in the developing mouse eyes and reached a peak at retinal maturity at 2 months. Western blots of retinal tissues revealed two major CERKL protein bands: 59 kDa (C1) and 37 kDa (C2). However, only C2 CERKL was found in the rat retinal rod outer segment (ROS) at level of that was not changed in light vs. dark adaptation. In the light-stressed retina, expression of *Cerkl* mRNA increased significantly, which was reflected in only on C2 CERKL protein. The CERKL protein localized prominently to the ganglion cells, inner nuclear layers (INL), retinal pigment epithelial (RPE) cells, and photoreceptor inner segments in the retinal sections. Nuclear localization of CERKL was not affected in RPE, INL and the ganglion cell layers in the light-stressed retina; however, the perinuclear and outer segment locations appear to be altered. In the *NeuroD1* knock-out mouse retina, the expression of *Cerkl* mRNA and protein decreased and that decrease also pertains to C2 CERKL. In conclusion, the retina had the highest level of *Cerkl* mRNA and protein expression, which reached its maximum in the adult retina; CERKL localized to ROS and RPE cells and the light-adaptation did not change the level of CERKL in ROS; light-stress induced *Cerkl* expression in the retina; and its expression decreased in *NeuroD1* knock-out retina. Thus, CERKL may be important for the stress responses and protection of photoreceptor cells.

1. Introduction

The Ceramide kinase like (*CERKL*) gene is one of the newest members of the retinitis pigmentosa (RP) family. Mutations of *CERKL* are associated with recessive, nonsyndromic retinitis pigmentosa (RP26) with significant macular involvement during the early stages of the disease (Ali et al., 2008; Auslender et al., 2007; Bayes et al., 1998; Tuson et al., 2004). Although Bayes et al. (1998) described cases of what they called recessive RP with appreciated heterogeneity in the phenotype, they also reported that younger patients (age 23 and 24 years) had macular alteration and significant central

Abbreviations: CERKL, ceramide kinase like; CERK, ceramide kinase; SD, Sprague Dawley; qRT-PCR, quantitative RT-PCR; ROS, rod outer segment.

* Corresponding author. Department of Ophthalmology, OUHSC, Dean A. McGee Eye Institute, 608 SL Young Blvd., Oklahoma City, OK 73104, United States. Tel.: +1 405 271 8001x30009; fax: +1 405 271 8128.
E-mail address: mmandal@ouhsc.edu (N.A. Mandal).
[1] Current address: Department of Ophthalmology, Gavin Herbert Eye Institute, University of California-Irvine, CA 92697, United States.

0014-4835/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.exer.2012.10.014

scotoma, which may indicate an early macular phenotype (Bayes et al., 1998). In 2004, Tuson et al. identified this gene and its mutation within members of the same family (Tuson et al., 2004). All affected individuals were homozygous for a nonsense mutation (R257X; CGA→TGA) in exon 5. The gene was named '*Ceramide kinase like*' based on its homology with *Ceramide kinase* (*CERK*) (Tuson et al., 2004). From the discovery of new mutations and from further characterization of the phenotype of the previously identified mutations, *CERKL* mutations are now considered as the cause of cone-rod dystrophy (CRD), which progresses to an RP-like phenotype in advanced stages (Aleman et al., 2009; Avila-Fernandez et al., 2008; Littink et al., 2010; Tang et al., 2009).

CERKL was initially considered as a retinal ceramide kinase. However, no kinase activity so far has been identified for this protein. CERKL expression is highly complex; more than 20 transcripts, which may generate various protein products, have been found in human and mouse tissues (Garanto et al., 2011). Attempts have been made to generate *Cerkl* knock-out mice; however, the transcriptional complexity of the gene makes it challenging to develop knock-out mice completely ablated for CERKL function (Garanto et al., 2012). CERKL has been shown to be expressed in various cell types in the retina, and a cone-dominant expression in mouse photoreceptors supports the notion that cone cell death precedes rods in humans with the *CERKL* mutation (Vekslin and Ben-Yosef, 2010). CERKL is also expressed significantly in ganglion cells and patients with *CERKL* mutations is known to develop significant pathology in the inner retina (Aleman et al., 2009). Given this transcriptional complexity, the *CERKL* mutation pathology is also complex.

In this study, we analyzed the expression and tissue distribution of *Cerkl* in rat tissues, confirmed its expression in mouse tissues and generated novel data on its expression in embryonic and developing mouse eyes to gain a better understanding of the role of this gene in the retina during embryogenesis and development. Because CERKL has previously been speculated as a retinal CERK (ceramide kinase), we performed a side-by-side comparative analysis of the expression of *Cerk* in every tissue and at developing stages. In a recent study, Nevet et al. (2012) showed an interaction between CERKL and neuronal calcium sensor (NCS) proteins, including guanylate cyclase activating protein 1 (GCAP1), GCAP2, and recoverin in the photoreceptor cells. We compared expression of these genes with *Cerkl* and *Cerk* expression in developing eye tissues. From previous *in vitro* studies, CERKL was attributed to have a protective effect against oxidative stress (Tuson et al., 2009). We used the light-stressed rat retina model in which photoreceptor cell death occurs by oxidative stress and measured the expression of the *Cerkl* gene and its protein and determined the localization of CERKL protein to understand whether CERKL is involved in retinal protection against stress.

We further analyzed expression of CERKL in the *NeuroD1* knock-out mouse retina. BETA2/NeuroD1 is a neuronal transcription factor; it is highly expressed in the developing retina, its genomic location is immediately adjacent to the 5′ of *CERKL*, and it is conserved in all known mammalian species (Cho et al., 2007). Interestingly, *NeuroD1* knock-out mice develop a characteristic retinal degeneration phenotype; both rod and cone cells begin to degenerate during the very early postnatal days (Pennesi et al., 2003). We speculated that these linked genes may also be functionally related.

2. Materials and methods

2.1. Animal and tissue collection

All procedures were performed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the University of Oklahoma Health Sciences Center (OUHSC) Guidelines for Animals in Research. All protocols were reviewed and approved by the Institutional Animal Care and Use Committees of the OUHSC, the Dean A. McGee Eye Institute (DMEI) and the University of Texas M. D. Anderson Cancer Center. All animals were maintained on a 12-h dim cyclic light (5–10 lux at the cage level)/12-h dark cycle, and tissues were collected at the end of a dark cycle at the appropriate age. We collected the forebrain, hindbrain, retina, eyecup containing RPE–choroid–sclera (eyecups = RPE + choroids + sclera; no retina), heart, lung, liver, spleen, kidney, skin, testis, and ovary from adult Sprague Dawley (SD) rats. Mouse eyeballs (6–8) were collected from embryonic day E15 and E18, and postnatal days P1, P3, P7, P15, P30, P60, P90, P210 to study the expression of the genes of interest in the whole eye. To study the distribution of this gene expression in mouse eye tissue, the distal-most 3-mm optic nerve (ON) cut from the scleral surface of the eyeballs, eyecups, retina, lens, iris–ciliary body (I–CB) and cornea were collected after dissection. Retinas from NeuroD1 knock-out mouse were collected at P30 for mRNA and protein expression studies.

For the immunofluorescence microscopy, adult albino (BALBc) mice and NeuroD1 knock-out mice (at P60) eyeballs were fixed in 4% paraformaldehyde and embedded in OCT. Cryosections (10 μm) were prepared for immunolocalization studies of the CERKL protein.

2.1.1. Light damage of albino rats

Eight- to ten week-old SD rats were exposed to damaging light (white cool light) for 6 h (9 AM–3 PM) at an intensity of 2700 lux following previously published protocols (Mandal et al., 2011, 2009). After light damage, the retinas and eyecups were harvested at select time points (0 h, 3 h, 6 h, 12 h, and 24 h after the completion of light-damage), snap frozen in liquid N_2, and prepared for RNA and protein analysis. For studying the localization of the CERKL protein in the light-exposed retina, dark-adapted non-light-damaged (NLD) and light-damaged (LD) eyes at 0 h and 24 h after LD were harvested, fixed in 4% paraformaldehyde, embedded in OCT and sectioned.

2.2. RNA isolation and cDNA synthesis for quantitative RT-PCR (qRT-PCR)

RNA was isolated from all of the collected tissues using TRIzol (Invitrogen) reagent following the manufacturer's protocol. First-strand cDNA synthesis was carried out (SuperScript II First-Strand Synthesis System; Invitrogen) for qRT-PCR. Primers for qRT-PCR were designed in such a way that they spanned at least one intron, which eliminated the possibility of amplification from residual genomic DNA contamination. Degenerative forward and reverse primers for qRT-PCR were designed to amplify both mouse and rat *Cerkl* sequences. As *Cerkl* is known to have several transcripts (Garanto et al., 2011; Tuson et al., 2009), the primers were selected from exon 11 (forward) and exon 12 (reverse) of the known protein encoding the *Cerkl* transcript (ensemble.org: Cerkl-001 ENSMUST00000143974; NCBI CCDS database: Mouse CCDS set: CCDS38157). These two exons are common in almost all of the known transcripts of *Cerkl* (Garanto et al., 2011), so that the amplification can represent the total *Cerkl* expression. Primer pairs were forward: 5′-AACAATGGAAGCATGGCTCT-3′; reverse, 5′-CTCCTGTGGGCTGTATC-CAT-3′ for *Cerkl* and forward: 5′-GTCCTTCCTCCCAGCACAG-3′; reverse, 5′-GCACTTCCGGATAAGGATGA-3′ for *Cerk*. The sequence of primers used for other retinal genes will be obtained from the corresponding author upon request. Quantitative PCR (using iQ SYBR Green Supermix; Bio-Rad, Hercules, CA) and melt-curve analysis (using iCycler; Bio-Rad) were performed. Mean values (±SD) were