Experimental Eye Research 152 (2016) 34–42

Contents lists available at ScienceDirect

Experimental Eye Research

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

Research article

Role of transcription factor *Tgif2* in photoreceptor differentiation in the mouse retina

Hiroshi Kuribayashi [a], Asano Tsuhako [a], Mio Kikuchi [b], Nobuaki Yoshida [b], Hideto Koso [a], Sumiko Watanabe [a, *]

[a] Division of Molecular and Developmental Biology, Institute of Medical Science, University of Tokyo, Tokyo, Japan
[b] Laboratory of Developmental Genetics, Center for Experimental Medicine and Systems Biology, Institute of Medical Science, University of Tokyo, Tokyo, Japan

ARTICLE INFO

Article history:
Received 29 February 2016
Received in revised form
9 September 2016
Accepted in revised form 13 September 2016
Available online 14 September 2016

Keywords:
Retina
Differentiation
Transcription factors
Photoreceptors
Cone

ABSTRACT

5′TG3′-interacting factors (TGIFs) function as transcriptional repressors. Defects in TGIFs cause severe abnormalities in the developing brain and face. We found that *Tgif2* was highly expressed in the mouse retina at early stages of development and examined its role in retinal development. Knockdown of *Tgif2* in retinal explants at E14 using shRNA (sh-*Tgif2*) resulted in a decreased number of rod photoreceptors, whereas the number of cone photoreceptors increased without perturbation of cell proliferation and apoptosis. Concomitantly, the expression levels of photoreceptor-related genes were decreased in sh-*Tgif2*-introduced retinal explants. To examine the *in vivo* effects of Tgif2 overexpression, we generated *Tgif2* conditional knock-in mice (*Tgif2*cKI). Although retinal cell differentiation, based on the relative proportions of retinal subtypes in the mature retina, was not affected, we observed abnormal localization of cone photoreceptor cell nuclei in the outer nuclear layer of the *Tgif2*cKI retina. However, electrical retinography suggest that cones in *Tgif2*cKI were functionally equivalent to those of wild mice. Our study revealed that Tgif2 participates in photoreceptor cell differentiation in the early stages of retinal development and regulates proper subretinal localization of the cone photoreceptors.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

The vertebrate retina consists of six types of neurons and Müller glia. In mice, these major retinal cell types are generated from common retinal progenitor cells between embryonic day (E) 11 and postnatal day (P) 10 in a conserved temporal order (Marquardt and Gruss, 2002). Retinal ganglion cells, amacrine cells, horizontal cells and cone photoreceptors differentiate primarily before birth. Bipolar cells, rod photoreceptors and Müller glia are largely generated after birth. A large number of studies have shown that the well-ordered retinal cell differentiation is regulated by the coordinated action of transcription factors (Cepko, 1999; Harris, 1997). Regulation of photoreceptor differentiation has been particularly well studies, which has revealed a network of several transcription factors involved in photoreceptor fate specification (Bizezinski and Reh, 2015).

5′TG3′-interacting factors (TGIFs) belong to the three-amino-acid-loop-extension (TALE) family of homeodomain proteins and consist of two closely related genes, *Tgif1* and *Tgif2* (Imoto et al., 2000). TGIFs were initially identified based on their ability to bind to a retinoid X receptor response element in retinol-binding protein II promoter (Bertolino et al., 1995). TGIFs bind to DNA directly via a thymine/guanine-containing site and function as transcriptional repressors (Bertolino et al., 1995). Further research has demonstrated that TGIFs regulate transcription through various mechanisms by interacting with other transcriptional modulators, such as histone deacetylase (HDAC), Sin3a transcriptional corepressor, and C-terminal binding protein (CtBP) (Melhuish et al., 2001; Wotton et al., 1999b). Modulation of various signaling patterns by TGIFs has been reported; for example, TGIFs are involved in tumorigenesis by repressing TGF-β signaling (Seo et al., 2006) through an interaction with SMAD proteins (Wotton et al., 1999a). More recently, the involvement of Wnt signaling modules in TGIF activity during mammary tumorigenesis was demonstrated (Razzaque and Atfi, 2015). Furthermore, we showed that *Tgif2* is

* Corresponding author. Division of Molecular and Developmental Biology, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.
E-mail address: sumiko@ims.u-tokyo.ac.jp (S. Watanabe).

http://dx.doi.org/10.1016/j.exer.2016.09.005
0014-4835/© 2016 Elsevier Ltd. All rights reserved.

associated with the Sonic Hedgehog subtype of medulloblastoma (Koso et al., 2014).

Deletion of *Tgif1* in mice causes defects in placental development (Bartholin et al., 2008), as well as brain developmental defects, such as holoprosencephaly (HPE), microcephaly, and exencephaly (Kuang et al., 2006); however, the phenotype depends on genetic background. Similarly, heterozygous mutation of *TGIF1* in humans leads to HPE (Gripp et al., 2000). These findings support the idea that TGIF plays a pivotal role in mammalian brain development. Conditional knockout of both *Tgif1* and *Tgif2* using *Sox2*-cre causes HPE-like developmental anomalies and defective eye field separation, which are phenotypes reminiscent of *Shh*-null embryos (Taniguchi et al., 2012). On the other hand, *TGIF2* mutations have not been reported in HPE patients (El-Jaick et al., 2007).

Although the role of TGIFs in brain development has been relatively well characterized, there is only limited knowledge of the function of TGIFs in the retina. Previously, we reported that *Tgif1* and *Tgif2* were strongly expressed in retinal progenitors during mouse retinal development, and ectopic expression of *Tgif1* or *Tgif2* in retinal explants resulted promoted amacrine cell differentiation (Satoh and Watanabe, 2008). Meanwhile, *Tgif1*-knockout mice showed no significant abnormalities in retinal structure or retinal cell differentiation (Satoh and Watanabe, 2008), suggesting that the activities of Tgif1 and Tgif2 in retinal development are redundant or that Tgif2 plays a more dominant role than Tgif1 in retinal development.

In this study, we evaluated the function of Tgif2 using sh-RNA mediated knockdown of Tgif2 in mouse retinal explant cultures and, *in vivo*, using a newly generated *Tgif2* over-expressing mouse. We found that the level of *Tgif2* influences the photoreceptor fate determination of retinal progenitor cells (RPCs) in the early stages of retinal development. Furthermore, *Tgif2* regulates the subretinal distribution of cone photoreceptors in the late stages of retinal development.

2. Materials and methods

2.1. Mouse

All experiments were approved by the Animal Care Committee of the Institute of Medical Science, the University of Tokyo and conducted in accordance with the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in ophthalmic and vision research. *Dkk3-cre* transgenic mice (*Dkk3-cre*) were provided by Dr. Takahisa Furukawa (Sato et al., 2007). They were bred with *CAG-LSL-Tgif2* transgenic mice to obtain double transgenic mice (*Dkk3-cre; CAG-LSL-Tgif2*), which were denoted as *Tgif2*cKI mice. Generation of *CAG-LSL-Tgif2* transgenic mice is described in the following section. The genetic background of these animals was mixed. Institute of Cancer Research (ICR) mice were obtained from Japan SLC Co.

2.2. Generation and characterization of CAG-LSL-Tgif2 knock-in mice

Mice with transgenic expression of *Tgif2* were generated by targeting of the *Rosa26* locus with the CTV vector (*R26-CAG*-STOP-*eGFP*-TV), a kind gift from Dr. Yoshiteru Sasaki (Calado et al., 2012), containing mouse *Tgif2* cDNA preceded by a *lox*P-flanked stop cassette. Thus, the transgene transcription in this construct is controlled by the *CAG* promoter (*CAG-LSL-Tgif2*). The *CAG-LSL-Tgif2* targeting vector was linearized and transfected into E14.1 129Sv-derived embryonic stem (ES) cells. G418 resistant ES cell clones were selected and analyzed for homologous recombination using Southern blot hybridization of *Eco*RI-digested genomic DNA (Fig. 3A–D). Probes were PCR amplified with the following primers. Probe 1 (5′ probe) F: 5′-tggagtaggcaatacccagg-3′, R: 5′-cacagcctcttctttaggcg-3′; Probe 2 (3′ probe) F: 5′-ggcactgtt-catttgtggtg-3′. R: 5′-gtgcctgtggaggctagaag-3′. Correctly targeted ES cell clones were injected into C57BL/6 blastocysts to produce chimeric mice. The chimeras were then mated to C57BL/6 mice. Germline transmission in agouti offspring were confirmed by PCR. PCR reactions using the following three primers generated 293-bp and 415-bp products for the knock-in and the wild-type alleles, respectively. F: 5′-gttatcagtaagggagctgcagtgg-3′. R1: 5′-aagaccgc-gaagagtttgtcctc-3′. R2: 5′-ggcggatcacaagcaataataacc-3′. For all the experiments, we prepared samples from at least three independent mice of each genotype, and counted marker positive cell number from 2–4 sections in each sample. Then, average and standard deviation were calculated.

2.3. Plasmid construction

For the construction of shRNA expression vectors, the target sequence (5′-ccgtttactttgcttagttttca-3′) for sh-*Tgif2*, (5′-gtgca-cattccaacctattcaaa-3′) for sh-*Tgif2*_2nd, and (5′-tagaaa-caaagggttttcttttc-3′) for sh-*Tgif1* were selected by using siDirect. BLAST analysis showed no homology between the target sequence and any other sequence in the mouse genome database. As for construction of control shRNA plasmids, scrambled sequences for sh-*Tgif2* (5′-atgtctgttacgtgttattctgt-3′) or sh-*Tgif1* (5′-atgta-catgtattggattaggat-3′) were used. The shRNA vectors were constructed as described previously (Satoh et al., 2009). To trace plasmid transfected cells, we co-transfected an EGFP expressing plasmid (p*CAG-EGFP*) with shRNA expression plasmid. For the overexpression of *Tgif2*, full-length *Tgif2* (Satoh et al., 2009) was inserted into p*CAG* vector by using *Eco*RI and *Not*I sites.

2.4. Examination of efficiency and specificity of sh-constructs

NIH3T3 cells were seeded on 6 well plate ($1 \times 10e5$ cells/well) one day before transfection, and sh-*Tgif1* and sh-*Tgif2* constructs with p*CAG-EGFP* were transfected into the cells by using GeneJuice transfection reagent (EMD Millipore). Cells were harvested after 48 h of culture, and total RNA was extracted by Sepasol-RNA (Nacalai), and qPCR to detect *Tgif1* and *Tgif2* was performed to examine efficiency and specificity of sh-constructs (Supplemental Fig. 2A).

2.5. Electroporation and retinal explant culture

Retinal explant culture and *in vitro* electroporation were performed as described previously (Iida et al., 2011; Tabata et al., 2004). Briefly, the total amount of plasmids used for electroporation was 100 μg (80 μg of p*U6*-sh-*Tgif2*, −sh-*Tgif1* or p*U6*-control plasmid and 20 μg of p*CAG-EGFP*) for each retina. Retinas dissected from E14.5 or E17.5 ICR mouse embryos were electroporated with plasmids, and cultured for 3, 5, 7 or 14 days according to the experimental condition. For rescue analysis, p*U6*-sh-*Tgif2* 60 μg, p*CAG-Tgif2* 20 μg, and p*CAG-EGFP* 20 μg were electroporated. For all the samples, we performed at least three independent electroporation and counted cells from 2–4 sections in each sample. Then, average and standard deviation were calculated.

2.6. Immunostaining and in situ hybridization

Immunostaining of frozen sections was done as described previously (Tabata et al., 2004). Briefly, freshly dissected retinas and retinal explants were fixed with 4% paraformaldehyde, and treated with 15% and 30% sucrose in this order, and embedded in the

Download English Version:

https://daneshyari.com/en/article/4011070

Download Persian Version:

https://daneshyari.com/article/4011070

Daneshyari.com