·Basic Research·

Expression of transforming growth factor-β type I receptor and transforming growth factor-β type II receptor in rat retina

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Abstract

• AIM: To quantitatively investigate the gene expression of transforming growth factor-β type I receptor (Tβ R I) and transforming growth factor-β type II receptor (Tβ R II) in rat retina.

• METHODS: Sprague-Dawley rats were chosen in this research. Gene expression was detected quantitatively by reverse transcription polymerase chain reaction (RT-PCR) analysis.

• RESULTS: The expression level of Tβ R I and Tβ R II were 0.00034 ± 0.00013 and 0.0001 ± 0.00005, respectively. The expression level of Tβ R I was obviously higher than that of Tβ R II in the rat retina with statistical significance (P<0.01). The ratio of Tβ R I /Tβ R II was 3.9± 1.7.

• CONCLUSION: Real time quantitative RT-PCR is an effective method to detect differential expression genes in retina. The expression change of Tβ R I and Tβ R II may play an important role in the pathogenesis of retinopathy, which need further investigation on its significance in the development of proliferation retinopathy.

• KEYWORDS: TGF-β receptor; quantitative reverse transcription polymerase chain reaction; gene expression; retina

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INTRODUCTION

Transforming growth factor-β (TGF-β) is one of the important members of TGF-β family, which control the development and homeostasis of most tissues in metazoan organisms, especially in retina. Recent studies showed that cytokines, including TGF-β, are involved in the occurrence of proliferative retinopathy, though the mechanism is unknown[1].

Researches over the past few years have led to the elucidation of a TGF-β signal transduction network. This network involves receptor serine/threonine kinases at the cell surface and their substrates, the SMAD proteins, which move into the nucleus where they activate target gene transcription in association with DNA-binding partners. Distinct repertoires of receptors, SMAD proteins, and DNA-binding partners underlie, in a cell-specific manner, the multifunctional nature of TGF-β and related factors. Mutations in these pathways are the cause of various forms of human disorders[2,3].

In the present study, quantitative real time reverse transcription polymerase chain reaction (RT-PCR) was developed to identify the differential expression of TGF-β type I receptor (TβR I) and TGF-β type II receptor(TβR II) genes in normal rat retina in order to discuss the possible functions of TGF-β and its receptors in the normal retinal homeostasis maintenance and pathogenesis of proliferation retinopathy.

MATERIALS AND METHODS

Experimental Animals Ten adult male Sprague-Dawley rats 200±25g were provided by Shanghai Laboratory Animal Center (Shanghai, China). The animals were housed in stainless steel cages and fed with standard rat chow and tap water. They were held in a room in a 12 hours:12 hours light:dark cycle with an ambient temperature of 18-22℃.

Total RNA in Retina and the First Strand of cDNA Preparation Eye globes of the experimental animals were enucleated at the 2nd week. The anterior of the globe and lens were removed and the retina were peeled off and immediately placed into liquid nitrogen for preservation.

Total RNA was isolated from frozen tissues in 0.5mL Trizol^{MT} reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Then, its purity and

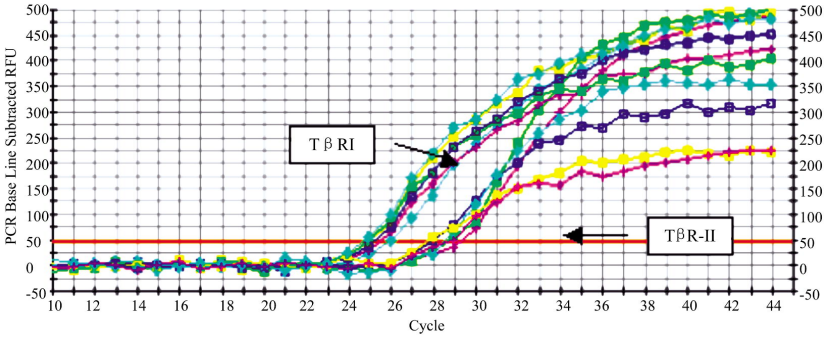

Figure 1 Quantitative reverse transcription polymerase chain reaction (QRT-PCR) curves for TGF-βRI/TGF-βRII in retina of normal rats

integrity were determined. First strand cDNA was reversibly transcribed from total RNA with SuperScript^{MT} II reverse transcriptase (Invitrogen).

With primer design based on the principle of quantitative real time RT-PCR primer design, 6-carboxyfluorescein (FAM) was selected as the fluorescent reporter group for the TaqMan fluorescent probe, and 6-carboxytetramethy-rhodamine (TAMRA) was selected is as the quenching group. Based on the PCR primer sequence of gene TβR I, TβR II and 18S of the rats, as well as the TaqMan probe sequence, the sequence of the primer synthesis is as follows: TβR I-F: 5′>ACC TTC TGA TCC ATC CGT T<3′;TβR I-R: 5′>CGC AAA GCT GTCAGC CTA G<3′; TβR I-TM: FAM 5′>CAG AGC TGT GAG GCC TTG AGA GTG<3′ TAMRA; TβR II-F: 5′>CCC TAC TCT GTC TGT GGA TGA<3′; TβR II-R: 5′>GAC GTC ATT TCC CAG AGT AC<3′; TβR II-TM: FAM 5′>CAG GTG GGA ACA GCG AGA TAC ATG G<3′ TAMRA;18S-F:5′> GTA ACC CGT TGA ACC CCA TT <3′;18S-R: 5′> CCA TCC AAT CGG TAG TAG CG <3′;18S-TM:FAM 5′> ATG GGG ATC GGG GAT TGC AAT <3′ TAMRA.

Real-time QRT-PCR Procedure and Data Analysis The real time QRT-PCR determination and analysis were performed as the methods introduced in previous articles[4]. Real-time QRT-PCR was performed in 96-well optical plates with the TaqMan Gold nuclease assay kit. The total reaction volume (50μL) consisted of 10×PCR buffer 5μL, 25mmol/L $MgCl_2$ 5μL, 5μmol/L 5'primer 1μL, 5μmol/L 3'primer 1μL, 5μmol/L TaqMan probe 1μL, 10mmol/L dNTP Mixture 2μL, templat 1μL, Taq enzyme 1μL and H_2O 33μL. The procedure of PCR was made with denaturing at 94℃ for 3 minutes followed by 35 cycles of reaction, including denaturing at 94℃ for 50 seconds, annealing at 58℃ for 50 seconds and elongation at 72℃ for 60 seconds, and a final bonus extension elongation at 72℃ for 5 minutes. Setting over in each cycle of degeneration, the process automatically recorded the final 10% of the average fluorescence value of cycle time in the last cycle at the end of the PCR. FAM-490 was chosen as the fluorescence type, and the procedures were set with excitation and emission spectra filters selected as 490nm and 530nm.

Data of background substrated and PCR baseline substrated were analyzed. Based on fluorescent curve and computed tomography (CT) values, the ratio of TβR I/18s and TβR II/18s were calculated respectively in the same tissue. The ratio of initial templates of TβR/18s was 2Ct18s-Ct TβR, as well as the expression of TβR in retina. In the same tissue, the ratio of initial templates of TβR I/TβR II was 2CTβR I - CTβR II, while CTβR I was the CT values of TβR I in retina, and CTβR II was the CT values of TβR II in retina. Results were expressed as mean±SD, and two-sample t-test was used for the comparison of the values.

RESULTS

Characteristics of the Real Time QRT-PCR Procedure for TβR Preliminary studies were performed to establish a reproducible and accurate QRT-PCR procedure. The linearity of generating a PCR product relative to cycle number was determined. The 35 cycles was selected as an appropriate cycle number for TβR I, the 33 cycles for TβR II receptor, and the 29 cycles for 18S. The three curves were parallel, indicating that the QRT-PCR assay could be used to quantitate mRNA levels (Figure 1).

Expression of TβRI and TβRII in Rat Retina The RNA of rat retina was integrative enough to be used for further QRT-PCR analysis. The expressions of TβRI and TβRII were 0.00034±0.00013 and 0.0001±0.00005, respectively. The expression of TβRI was obviously higher than that of TβR II. The mRNA level of TβRI was about 4 times of that of TβR II with statistical significance (P<0.01). The ratio of TβRI/TβR II was 3.9±1.7 (Figure 2).

DISCUSSION

Real-time QRT-PCR is the technique when trying to detect modifications in transcription levels in a reliable and reproducible manner. Expression of low-expressed gene was

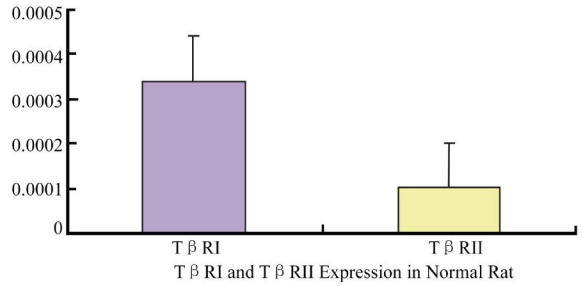

Figure 2 Expression of TGF-βRI and TGF-βRII in normal rats' retina

very difficult to exam with traditional method, such as Northern blot, half-quantitative PCR and Western blot. While with real-time QRT-PCR, very low level of gene expression in little tissue could be accurately determined.

TGF-β is an important mediator of cell growth, differentiation and proliferation, and plays a significant role in both normal and pathological retinal tissue. Under the functions of TGF-β and other cytokines, retinal pigment epithelial cells and retinal glial cells are relocated under the vitreous and retina, which stimulates the macrophages and fibroblasts to produce collagen and fibronectins. These might make the capillary basement membrane thickened and promote the formation of capillary lumen, and ultimately lead to the proliferative retinopathy[5]. The effects of TGF-β are elicited by activation of two types of membrane receptors containing serine/threonine kinase activity.

TGF-β receptors are a kind of surface membrane receptors, including three distinct forms (TβR I, TβR II and TβR Ⅲ). TβR I and TβR II are imperative in TGF-β signaling transduction system, while TβR Ⅲ is the foundation of the action. TβR I and TβR II are serine/threonine kinase receptors and they have common commencement action of TGF-β in the cytoplasm signal[6]. There are distinct structural characteristics of TβR I and TβR II, and their functions in TGF-β signaling pathways are also distinct. Due to its structural characteristics, TβR II itself has kinase activities and needs no activation, whereas TβR I does not have such natural activities, but after the formation of heteromeric complex of type I and type II receptors, the kinase domains of TβR I and TβR II become similar, and TβR I could be activated by TβR II, then the SMAD protein is activated, the signal is transducted to the nuclei, and the transcription of objective gene is regulated. Experimental studies have confirmed that TβR I is involved in the formation of extracellular matrix and that TβR II is involved in the cell proliferation [7]. In addition, it was found that the expression of both TβR I and TβR II could be detected on the retinal pigment epithelial cells and retinal glial cells of both normal rats and the rats of retinal neovascular disease with the methods of immunohistochemistry and *in situ* hybridization. Currently, TβR I and TβR II are considered to be essential in the TGF-β signaling pathways [8,9]. Both immunohistochemistry and *in situ* hybridization are methods for the locating and semi-quantitative determination of gene expression, QRT-PCR, however, could specifically and accurately detect gene expression level in retina.

In our study, we detected the gene expression level of TβR I and TβR II in retina by using QRT-PCR. The aim of this study was to determine the gene expression profile of TGF-β receptors in order to evaluate quantitative relations between the examined transcripts in retina. We found different expression of the TβR I and TβR II in rat retina. Such differential expression of TβR I and TβR II suggests that each of them may play a specific role in retinal tissue. The expression level of TβR I was obviously higher than that of TβR II in normal rats' retina with statistical significance ($P<0.01$). The ratio of TβR I/TβR II was 3.9±1.7. Our research shows that using QRT-PCR is an effective method to quantitatively detect differential expression genes in retina. The change of TβR I/TβR II expression may play an important role in the pathogenesis of retinopathy, which deserves further investigation on its significance in the development of proliferation retinopathy.

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