

Quantitative detection of the expression level of transforming growth factor - β 1 and - β 2 in rat retina with real time PCR

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Abstract

• **AIM:** To quantitatively detect the expression level of transforming growth factor- β 1 (TGF- β 1) and transforming growth factor- β 2 (TGF- β 2) genes in the retina of normal rat in order to determine the expression difference of TGF- β 1 and TGF- β 2 in retina.

• **METHODS:** The total RNA was isolated from which the first strand of cDNA was prepared. The mRNA levels of TGF- β 1 and TGF- β 2 were detected quantitatively by real time polymerase chain reaction (PCR).

• **RESULTS:** The mRNA levels of TGF- β 1 and TGF- β 2 were 0.0008 ± 0.0003 and 0.0378 ± 0.009 , respectively. Expression of TGF- β 2 was obviously higher than that of TGF- β 1 in rat retina with statistical significance ($t=12.37$, $P<0.001$). The ratio of TGF- β 2/TGF- β 1 was 55.00 ± 26.61 .

• **CONCLUSION:** Quantitative reverse transcription polymerase chain reaction (QRT-PCR) can specifically and accurately detect gene expression level in rat retina. In retina the TGF- β 2 gene is expressed more abundantly than TGF- β 1. It is suggested that TGF- β 2 play an important role in retina diseases.

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

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INTRODUCTION

The transforming growth factor- β (TGF- β) family of growth factors control the development and homeostasis of most tissues in metazoan organisms. TGF- β is a multifunctional cytokine, whose numerous cell and tissue activities include differentiation, proliferation, migration, extracellular matrix formation, hematopoiesis, angiogenesis, chemotaxis, immune functions and the induction of apoptosis^[1]. TGF- β is known to play a pivotal role in the development of proliferative vitreous retinopathy^[2]. In mammals, three isoforms of TGF- β have been identified: TGF- β 1, TGF- β 2 and TGF- β 3. Their biological functions seem to be different according to a study using knockout mice. The eye mainly expresses TGF- β 1 and TGF- β 2^[3].

In the present study, we choose transforming growth factor- β 1 (TGF- β 1) and transforming growth factor- β 2 (TGF- β 2) genes as the target genes to quantitatively detect their expression level in the retina of normal rats in order to determine the expression difference of TGF- β 1 and TGF- β 2 in retina, which will be able to provide the technical and substantial foundation for the study of gene in retina.

MATERIALS AND METHODS

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

Total RNA in Retina and the First Strand of cDNA Preparation The eyeglobes of the experimental animals were enucleated at the 2nd week. The anterior part of the globe and lens were removed and the retina was 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 manufacturer's instructions. Then its purity and integrity

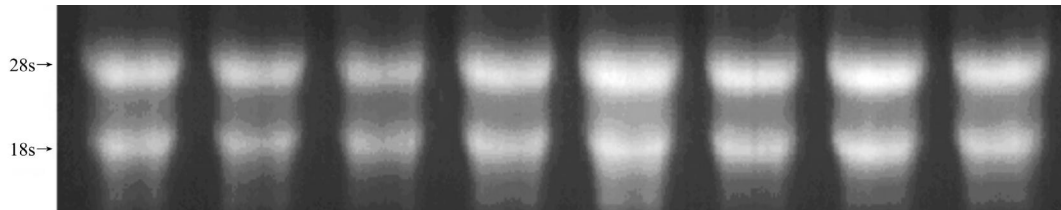


Figure 1 Total RNA retina of normal rats

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

Primers were designed on the basis of the principle of QRT-PCR, with 6-carboxyfluorescein (FAM) selected as the fluorescent reporter group for the TaqMan fluorescent probe and 6-carboxytetramethyl-rhodamine (TAMRA) as the quenching group. Based on the PCR primer sequence of gene TGF- β 1, TGF- β 2 and β -actin (ACTB) of rats, as well as the TaqMan probe sequence, the sequence of the primer synthesis is as follows: TGF- β 1-F: 5'-AAC TAC TGC TTC AGC TCC AC-3'; TGF- β 1-R: 5'-TGT GTC CAG GCT CCA AAT GTA-3'; TGF- β 1-TM: FAM5'-CAG AAG TTG GCA TGG TAG CCC TTG GG-3'TAMRA; TGF- β 2-F: 5'-ATG TGC AGG ATA ATT GCT GCC-3'; TGF- β 2-R: 5'-TGG TGT TGT ACA GGC TGA GG-3'; TGF- β 2-TM: 5'-FAM TGT TGT GTG TCT GAA CTC CAC AGA T-3'TAMRA; ACTB-F: 5'-GCC AAC ACA GTG CTG TCT G-3'; ACTB-R: 5'-CAC ATC TGC TGG AAG GTG G-3'; ACTB-TM: FAM5'-AGT ACT TGC GCT CAG GAG GAG C-3' TAMRA.

Quantitative Real-time PCR Procedure and Data Analysis The quantitative PCR determination and analysis were performed as the methods introduced in the reference^[4]. Real-time quantitative PCR was performed in 96-well PCR plates with the TaqMan Gold nuclease assay kit. Total reaction volume (50 μ L) was consisted of 10 \times PCR buffer 5 μ L, 25mmol/L MgCl₂ 5 μ L, 5 μ mol/L 5' primer 1 μ L, 5 μ mol/L 3' primer 1 μ L, 5 μ mol/L TaqMan probe 1 μ L, 10mmol/L dNTP Mixture 2 μ L, templat 1 μ L, Taq enzyme 1 μ L and H₂O 33 μ L. After 95 $^{\circ}$ C (for 3 minutes), samples were amplified for cycles of denaturation at 94 $^{\circ}$ C (50s), annealing at 60 $^{\circ}$ C (50s) and extending at 72 $^{\circ}$ C (50s), followed by bonus extending at 72 $^{\circ}$ C 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 TGF- β 1/ β -actin, and TGF- β 2/ β -actin were calculated respectively in the same tissue. The ratio of initials templates of TGF- β / β -actin was 2Ct β -actin-Ct TGF- β , as well as the expression of TGF- β in retina. In the same tissue, the ratio of initials templates of TGF- β 1/TGF- β 2 were 2Ct β 1-Ct β 2, while Ct β 1 was the CT values of TGF- β 1 in retina, and Ct β 2 was the CT values of TGF- β 2 in retina. Results were expressed as mean \pm SD and two-sample *t*-test was used for the comparison of the values.

RESULTS

RNA Isolation for Real-time PCR RNA extraction was highly purified and could be used for the experimental study. Since Strips of 18s and 28s were identified by 10g/L formaldehyde denaturing gel electrophoresis to be clear, and UV spectrophotometry measured A₂₆₀/A₂₈₀, the ratio was 1.8-2.0. The purity of RNA was very high without significant degradation of RNA (Figure 1).

Characteristics of the QRT-PCR Procedure for TGF- β

The parallel reactions of three PCR were determined and performed very well. The 40 cycles was selected as an appropriate cycle threshold number for TGF- β 1, 35 cycles for TGF- β 2, and 27 cycles for β -actin. For TGF- β 1, PCR amplification curve was determined mostly in 40 cycles, indicating TGF- β 1 expression was very low. These data indicated that expression of low-expressed gene was very difficult to exam with traditional method, such as Northern blot, half-quantitative PCR and Western blot. While with quantitative real-time PCR, very low level of gene expression in little tissue could be accurately determined (Figures 2, 3).

Expression of TGF- β 1 and TGF- β 2 in Rat Retina The mRNA levels of TGF- β 1 and TGF- β 2 were 0.0008 \pm 0.0003 and 0.0378 \pm 0.009, respectively. Expression of TGF- β 2 was

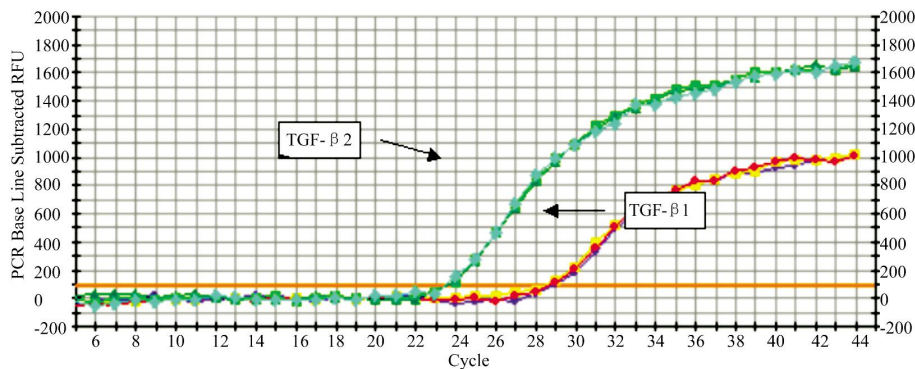


Figure 2 Quantitative reverse transcription polymerase chain reaction (QRT-PCR) curve for TGF-β2/TGF-β1 in retina of normal rat (one case)

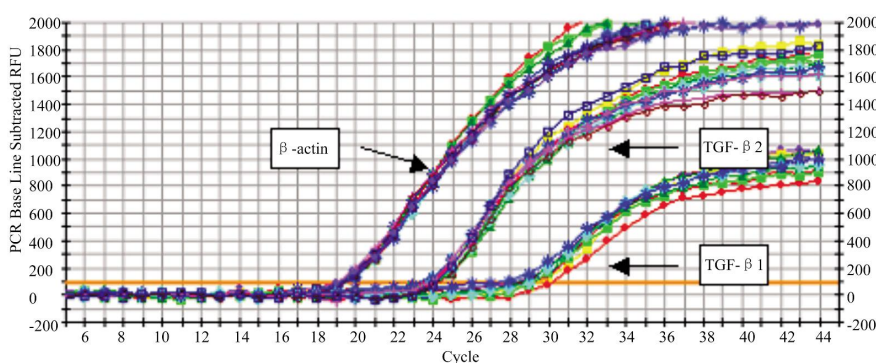


Figure 3 Quantitative reverse transcription polymerase chain reaction (QRT-PCR) curve for β-actin/TGF-β2/TGF-β1 in retina of normal rats (four cases). Threshold Value=4.43 (average SD) ×6.00 = 26.55

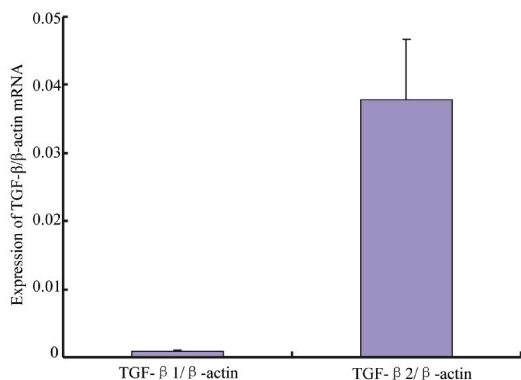


Figure 4 TGF-β1 mRNA and TGF-β2 mRNA expression in normal rats

obviously higher than that of TGF-β1 in rat retina with statistical significance ($t=12.37$, $P<0.001$). The ratio of TGF-β2/ TGF-β1 was 55.00 ± 26.61 (Figure 4).

DISCUSSION

TGF-β is an important mediator of cell growth, differentiation and proliferation. It plays a significant role in both normal and pathological retina tissue^[5, 6].

Of the five identified TGF-β isoforms, three are present in

mammals (TGF-β1, TGF-β2 and TGF-β3). Each of the TGF-β isoforms is encoded by a unique gene on the chromosome. The primary functions of the TGF-β isoforms are enhancing formation of the extracellular matrix and inhibiting proliferation of most cells^[7]. However, the quantitative relations between TGF-β1 and TGF-β2 isoforms in retina still remain unclear.

Since the previous study of TGF-β expression was examined with traditional methods, such as immunohistochemistry, Northern blot and Western blot. These methods were only half-quantitative analyzed. The expression of low-expressed gene was very difficult to exam. While with quantitative real-time PCR, very low level of gene expression in little tissue could be accurately determined.

QRT-PCR is the technique in detecting modifications in transcription levels in a reliable and reproducible manner. Nevertheless, there are some technical issues that must be taken into account, such as quality and quantification of the starting material, enzyme efficiency, and primer design. Different approaches have been proposed to normalize measurements of expression levels, but this is generally done

using an internal control gene, known as a reference gene or as housekeeping gene (HKG). With the assumption that this has a constant level of expression in the chosen tissue, it is not affected by the treatment and has no inter-individual variability. In addition, the reference gene and the target gene should have similar ranges of expression to avoid analytical problems.

This study showed the different gene expressions of the TGF- β 1 and TGF- β 2 in the rat retina with QRT-PCR. The mRNA level of TGF- β 2 was about 55 times of that of TGF- β 1 and there was statistic significance ($P < 0.001$). Such differential expression suggests that TGF- β may play a specific role in retina tissue. QRT-PCR could specifically and accurately detect gene expression level in rat retina. In retina the TGF- β gene was expressed more abundantly than TGF- β 1. It is suggested that TGF- β 2 play an important role in retina diseases.

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