

PPAR γ : the dominant regulator among PPARs in dry eye lacrimal gland and diabetic lacrimal gland

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Abstract

• **AIM:** To investigate the regulatory roles of the members of the peroxisome proliferator-activated receptor (PPAR) family in lacrimal gland dysfunction under conditions of desiccating stress or diabetes.

• **METHODS:** Quantitative polymerase chain reaction (qPCR) was used to examine the expression of PPARs in the cornea, conjunctiva, meibomian gland, and lacrimal gland in adult rats. The rats were divided into 3 groups: a control group, dry eye group, and diabetic group. The phenol red threads test, tear film break-up time (BUT) test and fluorescein staining were carried out to evaluate the development of dry eye. Based on bioinformatics research, qPCR was used to examine the expression level of PPAR γ , tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), sirtuin 1 (Sirt1), myeloid differentiation factor 88 (MyD88) and transforming growth factor- β (TGF- β) in the lacrimal glands.

• **RESULTS:** PPAR α and PPAR β/δ were mainly expressed in the conjunctiva and the lacrimal gland, respectively. However, PPAR γ was expressed in both the conjunctiva and lacrimal gland, at much higher levels than those measured for PPAR α and PPAR β/δ . Dry eye rats and diabetic rats both showed decreased tear secretion, shortened BUT, and increased corneal staining. Significant changes in gene expression were observed compared with the control group. In the lacrimal glands of dry eye rats and diabetic rats, expression of PPAR γ decreased ($P < 0.05$), expression

of Sirt1 also decreased ($P < 0.01$), whereas expression of TNF- α , IL-1 β , IL-6, MyD88, and TGF- β increased ($P < 0.05$).

• **CONCLUSION:** Among PPARs, PPAR γ might play a dominant role in the regulation of metabolic- and inflammatory-signaling pathways on the ocular surfaces and in lacrimal glands. Down-regulation of PPAR γ is highly relevant to lacrimal gland dysfunction under desiccating-stress and diabetic conditions. PPAR γ , thus, is a potential therapeutic target in the treatment of environment- or diabetes-induced dry eye diseases.

• **KEYWORDS:** dry eye; diabetes; PPAR γ ; rat; lacrimal gland

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INTRODUCTION

Dry eye is an ocular surface disorder that affects millions of people worldwide^[1]. It imposes a substantial social burden, influencing not only productivity in the workplace but also daily activities such as driving, reading, and recreation. There are many causes for dry eye, including overuse of electronic devices, low-humidity environments, medications, contact lens use and ocular surgery^[2]. Dry eye can also be a complication of eye diseases, including meibomian gland dysfunction^[3], lagophthalmos and ectropion, and decreased tear secretion associated with dysfunction of the lacrimal gland, or be associated with systemic diseases, such as Sjögren's syndrome and allergies^[4]. The prevalence of dry eye is expected to increase owing to increasing longevity. While diabetic retinopathy (DR) and diabetic cataracts are complications of diabetes, dry eye syndrome is also common^[5-8] in the rapidly increasing diabetic population worldwide. Patients with type 1 and type 2 diabetes often show more-severe dry eye symptoms than the patients without diabetes. The severity of dry eye in diabetic patients is closely related to the progression of diabetes^[9]. Common mechanisms in dry eye and diabetes include oxidative stress responses and inflammation, apoptosis, etc^[10].

Peroxisome proliferator-activated receptor (PPAR) is a member of the nuclear-receptor superfamily. It is divided into three subtypes: PPAR α , PPAR β/δ , and PPAR γ . PPARs are transcriptional factors participating in the regulation of glucose and lipid metabolism, and cell proliferation and differentiation. After ligand activation, PPARs can dimerize with the retinoid X receptor and then act in specific DNA sequences to regulate gene transcription downstream. Additionally, with other transcription factors, PPARs can suppress target-gene expression in a DNA-binding-independent way^[11].

It has been reported that PPAR γ is involved in many physiological and pathological processes of the ocular surface. Agonists of PPAR γ can promote wound healing after corneal injury and can block corneal fibrosis^[12]. In addition, studies confirm that agonists of PPAR γ can reduce the symptoms of meibomian gland dysfunction and the expression level of inflammatory cytokines^[13]. It is also known that PPAR γ is down-regulated in the conjunctiva in dry eye mice^[14]. However, there is limited literature on the relationship between PPAR γ and the lacrimal gland. *In vitro* studies have demonstrated that PPAR γ agonists can inhibit nitric oxide production in cultured lacrimal gland acinar cells^[15], but there have not been any *in vivo* experiments exploring the relationship between PPAR γ and the lacrimal gland in dry eye conditions. The lacrimal gland contributes multiple components to the tear film, and has been the object of much research, including research into many products that are now in clinical trials^[16]. Furthermore, research on the relationship(s) among PPAR γ , dry eye, and diabetes-associated dry eye is lagging-behind and is underrated. The purpose of the current study was to investigate the predominance in expression and function of the three subtypes of PPARs in ocular surface tissues and in the lacrimal gland, and, further, to discuss the common signal pathways related to PPAR γ in desiccating-stress dry eye conditions and diabetic conditions.

MATERIALS AND METHODS

Ethical Approval Institutional Animal Care and Use Committee of Tianjin Medical University (No. TJYY20190630012). Experiments were implemented in accordance with the guidelines of the National Institute of Health Guide for Care and Use of Laboratory Animals. We adhered to the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research.

Animals Adult Wistar rats were purchased from the Beijing Charles River Laboratory Animal Company Ltd. (Beijing, China) at 8 weeks of age and were maintained on a 12-hour light-dark cycle. All rats were provided with food and water *ad libitum*. In total, 24 male rats and 6 female rats were used in this study. Six male and 6 female rats were sacrificed as

soon as they were purchased to determine the expression of PPARs in the cornea, conjunctiva, meibomian gland, and lacrimal gland. Eighteen rats were randomly divided into three groups: a diabetic group, a dry eye group, and a control group. After 8wk, the rats were sacrificed and their lacrimal glands removed.

Diabetic Model and Dry Eye Model Type 1 diabetes was induced in rats by intravenous injection of 55 mg/kg streptozocin (STZ; Sigma, VA, USA) in 0.1 mol/L citrate buffer (pH 6.3; Solarbio, Beijing, China). Rats in the control group received only the buffer, through intravenous injection. Dry eye was induced in the dry eye group by putting the rats into intelligent drying bellows, which kept the ambient humidity below 45% with the fan blowing. Rats in the diabetic group and in the control group were put into a normal environment. Eight weeks later, for terminal studies, the rats were euthanized by cervical vertebra dislocation. Blood of the rats in the three groups was obtained from the tail vein after being established for 3d. We then measured blood sugar with OneTouch Ultra glucose test strips (Johnson & Johnson, Shanghai, China). The diabetic rats all had blood sugar values >30 mmol/L.

Phenol Red Threads Test The rats were restrained without anesthesia. Once the rat was calm, their lower eyelid was opened gently. Phenol red threads (Jingming, Tianjin, China) were put into the fornix of the lower eyelid using sterile forceps. The threads were removed after 30s. The length of the red threads was then recorded. All measurements were repeated three times.

Tear Film Break-up Time Test The rats were restrained without anesthesia. Once the rat was calm, their lower eyelid was opened gently and 1 mL of 10-g/L fluorescein sodium injection (Alcon Laboratories, Fort Worth, TX, USA) was dropped into the conjunctival sac. The rats were observed with a slit-lamp after 10 investigator-assisted blinks. The BUT was recorded as the number of seconds that elapsed between the last blink and the appearance of a black dot (dry spot). All measurements were repeated three times.

Fluorescein Staining of the Cornea Immediately after the break-up time (BUT) test, fluorescein staining tests were performed. The cornea of each rat was divided into four quadrants and each quadrant was graded. The total score for fluorescein staining was calculated as the sum of the scores of each quadrant. A score of 0 meant that the quadrant did not show any staining. A score of 1 was given if there were ≤ 30 stained points. A score of 2 was given for >30 stained points, without dispersive staining. A score of 3 was given for dispersive staining without plaque-like staining and a score of 4 was given for plaque-like staining.

Table 1 Primers for quantitative polymerase chain reaction

Gene	Forward 5'-3'	Reverse 5'-3'
TNF- α	5'-AGATGTGGAAGCTGGCAGAGG-3'	5'-CCCATTTGGGAAGCTTCTCCT-3'
IL-1 β	5'-CTGTGACTCGTGGGATGATG-3'	5'-GGGATTTGTGCGTTGCTTGT-3'
IL-6	5'-CCGGAGAGGAGACTTCACAG-3'	5'-ACAGTGCATCATCGCTGTTC-3'
IFN- γ	5'-AGGCCATCAGCAACAACATAAGTG-3'	5'-GACAGCTTTGTGCTGGATCTGTG-3'
TGF- β	5'-CAGAAGTTGGCATGGTAGCC-3'	5'-TGCTTCAGCTCCACAGAGAA-3'
Sirt1	5'-GCAGGTTGCAGGAATCCAAA-3'	5'-GGCAAGATGCTGTTGCAAAG-3'
ACE	5'-CACCGCAAGGTCTGCTT-3'	5'-CTTGGCATAGTTTCGTGAGGAA-3'
AGER	5'-CTCACAGCCAATGTCCCTAA-3'	5'-CCTTTGCCATCAGGAATCAG-3'
PPAR γ	5'-TGACTTGCCATATTTATAGCTGTCA-3'	5'-CGATGGGCTTCACGTTTCAG-3'
PPAR α	5'-GGCTCGGAGGGCTCTGTCATC-3'	5'-ACATGCACTGGCAGCAGTGGA-3'
PPAR β/δ	5'-CACCATGGAACAAAACTCATCTCAGAAGA GGATCTGGAGCAGCCACAGGAGGAAGC-3'	5'-TTAGTACATGTCCTGTAGATCTCC-3'
β -actin	5'-TCTGTGTGGATTGGTGGCTCTA-3'	5'-CTGCTTGCTGATCCACATCTG-3'

Reverse Transcription and Quantitative Real-time PCR

The rats were euthanized by cervical vertebra dislocation and the RNA from the cornea, conjunctiva, meibomian gland and lacrimal gland was isolated using TRIzol (Thermo Fisher Instruments, Waltham, MA, USA). Then, the RNase-free & DNase-free water -treated RNA was converted into cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Instruments). Polymerase chain reaction (PCR) primers for target and housekeeping-gene β -actin were designed using the National Center for Biotechnology Information (NCBI) Primer Blast and OligoCalc software. The primer sequences are listed in Table 1. Quantitative analysis of the expression of target and housekeeping genes was performed using a 384-well-plate system (Corning, New York, NY, USA) with a SYBR Green Fast Start 2X Master Mix (Roche, Mannheim, Germany) and gene expression was calculated using the $2^{-\Delta\Delta CT}$ method.

Bioinformatics Pathway Analysis A literature search was performed in the NCBI database. We opened the webpage “<https://www.ncbi.nlm.nih.gov/>”. Then we typed the phrase “PPAR and diabetic and dry eye” in the search box and selected “gene” as search scope. Genes related to “PPAR and diabetic and dry eye” were shown in the page. The results were used as input for further informatics searches in: the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, the Database for Annotation, Visualization and Integrated Discovery (DAVID), and the Search Tools of the Retrieval of Interacting Genes/Proteins (STRING) database. We opened the webpage “<https://david.ncifcrf.gov/>” and then clicked “Functional Annotation” in the right. In the webpage popped up, we input genes we got in NCBI database and selected “OFFICIAL_GENE_SYMBOL” as search scope. In the results we got, we chose “KEGG_PATHWAY” for further research. We accessed SRTING database by entering “[\[db.org/\]\(https://string-db.org/\)”. Then we input genes we got in NCBI database and selected “Multiple proteins” as search scope. We chose “Rattus norvegicus” in the pop-up webpage and finally we got pictures illustrated the interactions between these genes.](https://string-</p>
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Statistical Analysis The data were analyzed using SPSS version 22.0 software (IBM Corp., Armonk, NY, USA). The expression of genes, phenol red threads test scores, BUT, and fluorescein staining scores are all presented as the mean \pm standard deviation (SD). Repeated-measurement analysis of variance (ANOVA) was performed to compare the numerical values among different groups. A least-significant difference (LSD) was used as post hoc analysis. In all the experiments, *P*-values less than 0.05 were considered to be statistically significant.

RESULTS

Distributions of PPARs in Rat Cornea, Conjunctiva, Meibomian Gland, and Lacrimal Gland

Real-time PCR results showed that PPAR α was detectable in the cornea, conjunctiva, meibomian gland, and lacrimal gland of the rats. Among the four tissues, PPAR α was mainly expressed in the conjunctiva (Figure 1A). PPAR β/δ was only detected in the cornea and lacrimal gland, with higher expression in the lacrimal gland than in the cornea (Figure 1B). PPAR γ was detected in all four types of tissue, but was expressed more in the conjunctiva and lacrimal gland, than in the cornea and meibomian gland (Figure 1C). By comparing the expression levels of PPARs, we found that the expression of PPAR γ was much higher than that of PPAR α and PPAR β/δ in all the tissues. Of note, the expression of the three PPAR genes in male rats was slightly lower than in female rats, but the differences were not statistically significant. These findings suggest that among the PPAR families, PPAR γ might play a dominant regulatory role in the metabolic-signaling pathways on the surface of the eye.

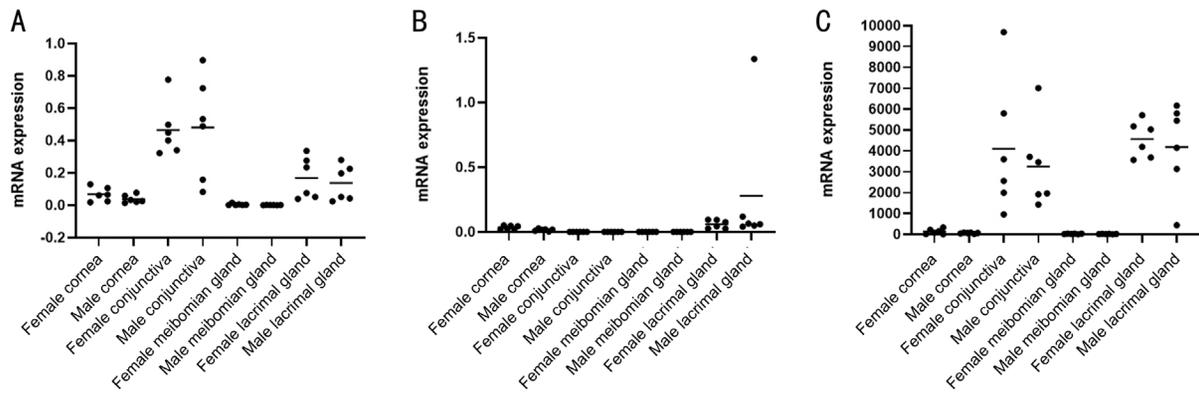


Figure 1 Real-time PCR of the distributions of PPAR α (A), PPAR β/δ (B), and PPAR γ (C) in rat cornea, conjunctiva, meibomian gland, and lacrimal gland ($n=6$ normal rats per gender) PPAR α : Peroxisome proliferator-activated receptor α ; PPAR β/δ : Peroxisome proliferator-activated receptor β/δ ; PPAR γ : Peroxisome proliferator-activated receptor γ .

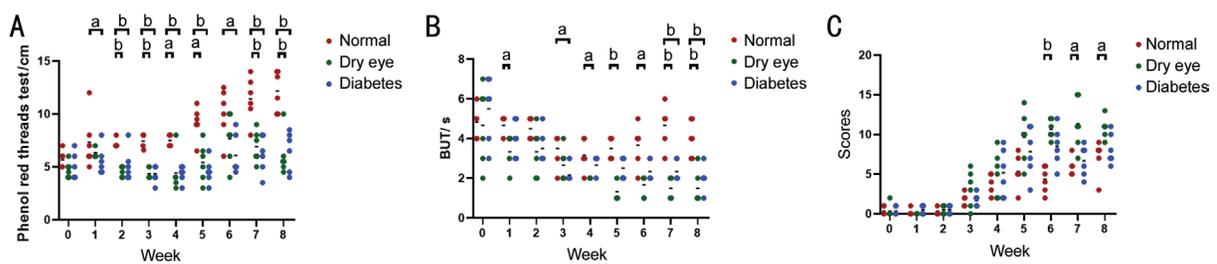


Figure 2 Results of phenol red threads test (A), BUT test (B), and fluorescein staining test (C) The aim of these tests was to assess the function of the lacrimal gland. ^a $P<0.05$ and ^b $P<0.01$ compared with the control group, $n=6$.

Objective Measures of Lacrimal Gland Function To assess the secretory function of the lacrimal gland, we performed phenol red threads tests, BUT, and corneal fluorescein staining tests on the three groups of rats every week. The phenol red threads test results from the rats in the dry eye group and the diabetic group decreased after the first week compared with the results from the control group (Figure 2A). Similarly, the BUT results showed downward trends in the dry eye group and the diabetic group compared with the control group after one week (Figure 2B). The fluorescein staining scores showed a general upward trend in the rats in the dry eye and diabetic groups, compared with those of the control group after 4wk of stress challenges. However, there were no consistent statistically significant differences at different time points due to the variability in the data (Figure 2C). These data show that basal tear secretion decreased, the stability of tear film decreased, and the corneal injury became more severe in both the dry eye group and the diabetic group compared with the control group, indicating the development of lacrimal gland dysfunction.

Role of PPAR γ in Dry Eye and Diabetic Ocular Surface Changes, Based on KEGG Gene Pathway Since the expression of PPAR γ was predominant on the ocular surface of all PPARs, PPAR γ might play a major role in the regulation of PPAR signaling pathways downstream. To explore the potential regulatory role of PPAR γ in the dry eye and diabetic ocular surface changes, we searched the NCBI database

using the phrase “PPAR and diabetes and dry eye”. The 12 most-related genes were: tumor necrosis factor- α (TNF- α), interleukin 1 β (IL-1 β), interleukin 6 (IL-6), angiotensin I-converting enzyme (ACE), advanced glycation end-product receptors (AGER), sirtuin 1 (Sirt1), myeloid differentiation factor 88 (MyD88), transforming growth factor- β (TGF- β), PPAR γ , interferon- γ (IFN- γ), toll-like receptor 4 (TLR4), and vascular endothelial growth factor A (VEGFA). These 12 were selected and input into the KEGG database, to explore the pathways. Additionally, the 12 genes were input into the DAVID database^[17] and the results showed that they were enriched in 38 pathways. Twenty-five pathways were picked based on $P<0.05$ (Table 2). Nine genes appeared on the 25 pathways; 6 of them (IL-6, MyD88, TNF, IFN- γ , TLR4, and TGF- β) appeared more than 10 times. Based on the pathways highly related to diabetes and dry eye diseases, five pathways were chosen for further research (Table 3). Among them, the TGF- β and NF-kappa B signaling pathways shared the same genes and the interactions overlapped with the other three pathways. Therefore the hypoxia-inducible factor 1 (HIF-1) pathway (Figure 3A), toll-like receptor pathway (Figure 3B), and cytokine-cytokine receptor interaction pathway (Figure 3C) were chosen for comprehensive analysis. The HIF-1 signaling pathway involved four of the six genes (IL-6, IFN- γ , VEGFA, and TLR4). IL-6, IFN- γ , and TNF were involved in both the toll-like receptor signaling pathway and the cytokine-

Table 2 Twenty-five pathways enriched with the genes of interest ($P < 0.05$)

Order	Pathways	Genes	<i>P</i>
1	Chagas disease (American trypanosomiasis)	7	8.96E-10
2	Malaria	6	2.29E-09
3	Rheumatoid arthritis	6	3.02E-08
4	Inflammatory bowel disease (IBD)	5	6.36E-07
5	Leishmaniasis	5	6.80E-07
6	Tuberculosis	6	1.04E-06
7	Amoebiasis	5	5.47E-06
8	Toxoplasmosis	5	5.68E-06
9	African trypanosomiasis	4	8.62E-06
10	Hepatitis B	5	2.41E-05
11	Influenza A	5	3.58E-05
12	Legionellosis	4	5.35E-05
13	Pertussis	4	8.74E-05
14	Hypertrophic cardiomyopathy (HCM)	4	1.07E-04
15	Salmonella infection	4	1.19E-04
16	HIF-1 signaling pathway	4	2.04E-04
17	Toll-like receptor signaling pathway	4	2.30E-04
18	Measles	4	5.06E-04
19	Osteoclast differentiation	4	5.18E-04
20	Graft-versus-host disease	3	8.07E-04
21	Herpes simplex infection	4	0.001293
22	Proteoglycans in cancer	4	0.001465
23	Cytokine-cytokine receptor interaction	4	0.001723
24	TGF- β signaling pathway	3	0.004089
25	NF-kappa B signaling pathway	3	0.005429

TGF- β : Transforming growth factor- β ; HIF-1: Hypoxia-inducible factor 1.

Table 3 Genes of interest from Table 2

Order	Pathways	Genes	<i>P</i>
1	HIF-1 signaling pathway	IL-6, IFN- γ , VEGFA, TLR4	2.04E-04
2	Toll-like receptor signaling pathway	IL-6, MyD88, TNF, TLR4	2.30E-04
3	Cytokine-cytokine receptor interaction	IL-6, TNF, IFN- γ , TGF- β 1	0.001723
4	TGF- β signaling pathway	TNF, IFN- γ , TGF- β 1	0.004089
5	NF-kappa B signaling pathway	MyD88, TNF, TLR4	0.005429

HIF-1: Hypoxia-inducible factor 1; IL-6: Interleukin 6; IFN- γ : Interferon- γ . MyD88: Myeloid differentiation factor 88; VEGFA: Vascular endothelial growth factor A; TNF: Tumor necrosis factor; TLR4: Toll-like receptor 4; TGF- β 1: Transforming growth factor- β 1.

cytokine receptor interaction pathway. TLR4 and MyD88 were found in the toll-like receptor signaling pathway, whereas TGF- β 1 was in the cytokine-cytokine receptor interaction pathway.

We then used the STRING database^[18] to predict the potential interactions of PPAR γ among these 12 genes (Figure 3D), and generated the interaction map. The edges of the nodes in the diagram reflect the inter-dependent relationships between the genes. Connecting lines with different colors, between any two proteins, indicate that the experiment verified or predicted interactions between that pair of proteins. Multiple lines

between two proteins suggest various potential interactions. This protein-interaction network illustrates that PPAR γ actively regulates the functions and activities of TLR4, MyD88, IL-1 β , IL-6, TNF, IFN- γ , TGF- β , and VEGFA.

PPAR γ and Sirt1 Down-regulated in the Lacrimal Gland of Diabetic and Dry Eye Rats By the eighth week, the expression of PPAR γ and Sirt1 (Figure 4) had decreased in both the diabetic group and the dry eye group ($P < 0.05$), relative to the control group. This suggested that PPAR γ and Sirt1, the two major regulators in the metabolic pathways and key protectors against inflammation, might share common

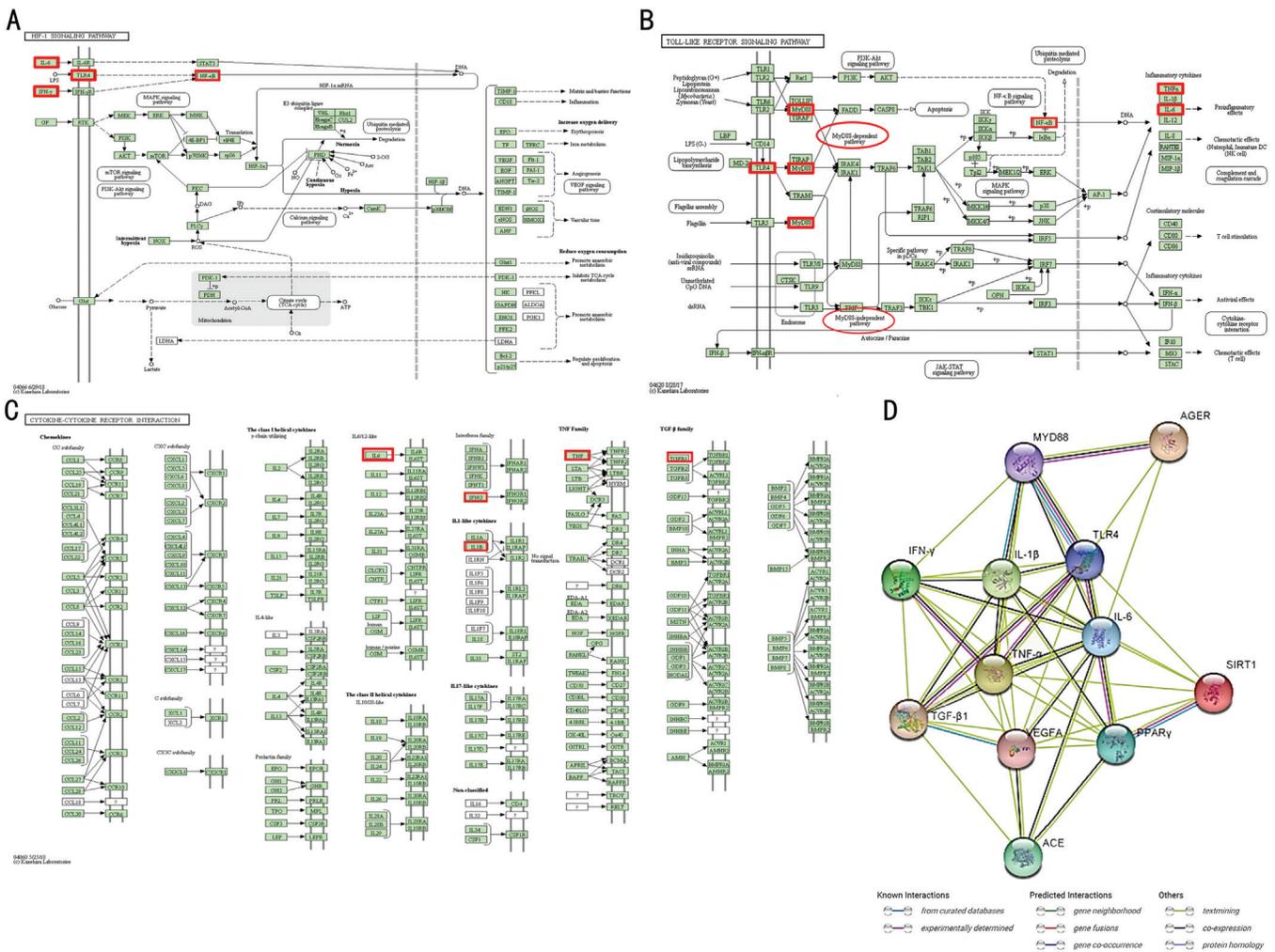


Figure 3 Genes of interest in HIF-1 signaling pathway (A), toll-like receptor pathway (B), and cytokine-cytokine receptor interaction pathway (C), as generated by the DAVID database. The STRING database illustrates the interactions between the 12 genes. The different line colors indicate different potential interactions (D).

protective roles in the development of diabetic ocular surface pathology and dry eye.

Pro-inflammatory Cytokines Up-regulated in the Lacrimal Gland of Diabetic Rats and Dry Eye Rats To verify the potential correlation in lacrimal gland dysfunction under desiccating-stress conditions and diabetes, which is probably regulated by PPARs, the mRNA expression levels of related cytokines were checked in the lacrimal glands of rats at 8wk after the models were established. However, the expression levels of IFN-γ, TLR4, and VEGFA were very low and barely detectable in the lacrimal glands using reverse transcription-polymerase chain reaction (RT-PCR; data not shown). ACE and AGER were abundantly expressed by lacrimal gland fibroblasts, but showed little correlation with the development of dry eye (data not shown). Finally, IL-6, IL-1β, TNF-α, TGF-β, and MyD88 were chosen for further investigation. The PCR data showed that mRNA expressions of IL-6 (Figure 5A), IL-1β (Figure 5B), TNF-α (Figure 5C), TGF-β (Figure 5D), and MyD88 (Figure 5E) were increased in both the dry

eye and diabetic groups compared with the control group. Altogether, these data indicate that, the decreased expression of PPARγ and Sirt1 under stress conditions was highly relevant to the increased expression of the IL-6, IL-1β, TNF-α, TGF-β, MyD88 genes. It suggested a potential regulation of inflammatory pathways by PPARγ and Sirt1.

DISCUSSION

PPARs are ligand-activated transcription factors of the nuclear-receptor superfamily, comprising three members: PPARα, PPARγ, and PPARβ/δ. They play essential roles in energy homeostasis and in metabolic function, providing regulatory effects in conditions such as dyslipidemia, diabetes, and inflammation. However, the distribution and metabolic regulatory roles of these three subtypes of PPAR are different. PPARα is involved in the regulation of energy homeostasis, PPARγ determines insulin sensitization and glucose metabolism, whereas activation of PPARβ/δ enhances fatty acids metabolism^[19]. Prominent expression of PPARs is specific to certain organs and tissues, in which the dominant

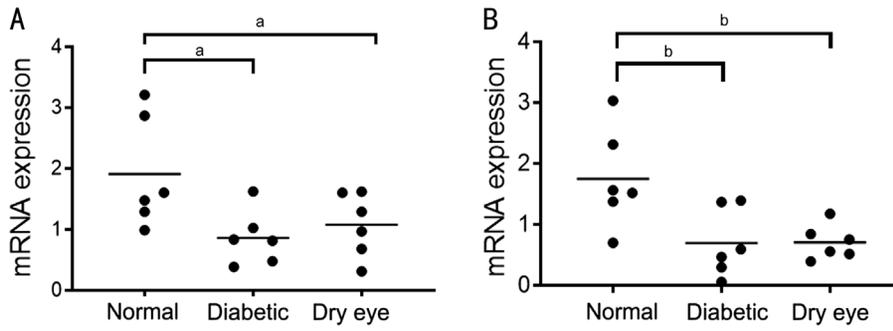


Figure 4 Real-time PCR analysis of PPAR γ (A) and Sirt1 (B) expression in the lacrimal gland of normal rats, diabetic rats, and dry eye rats ^a $P < 0.05$ and ^b $P < 0.01$ compared with the control group, $n = 6$.

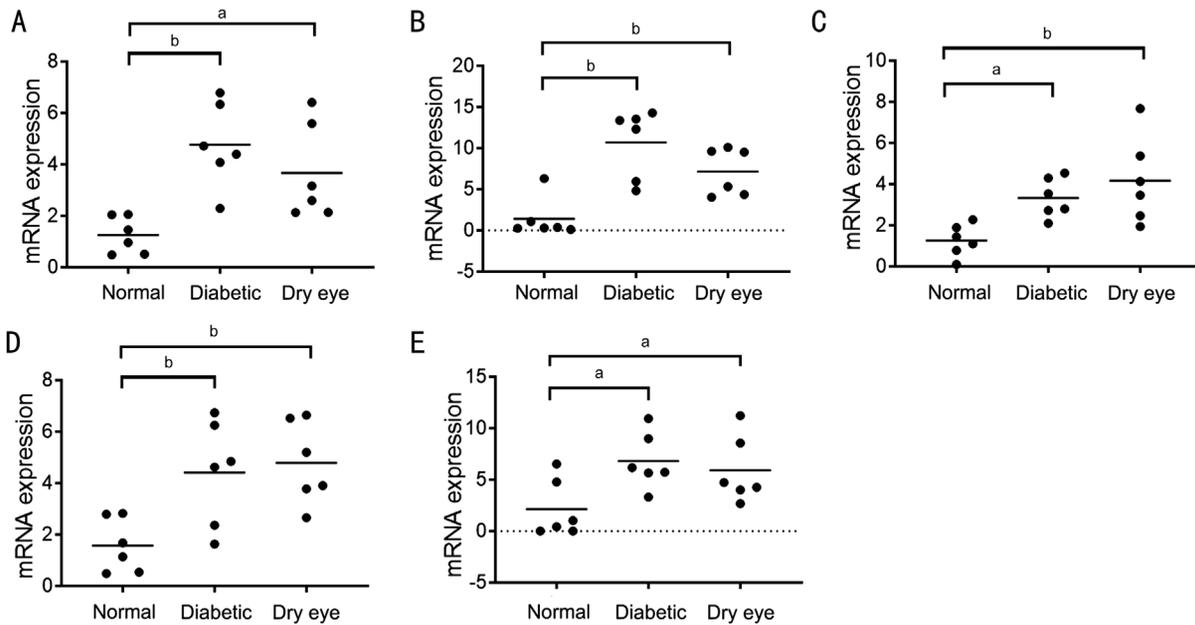


Figure 5 Real-time PCR analysis of IL-6 (A), IL-1 β (B), TNF- α (C), TGF- β (D), and MyD88 (E) expression in the lacrimal gland of diabetic rats and dry eye rats ^a $P < 0.05$, ^b $P < 0.01$ compared with control group, $n = 6$.

regulatory roles of PPARs are not necessarily the same. Studies by different groups have confirmed that PPAR α is mainly expressed in liver and retina. The expression level of PPAR α was much higher than PPAR β/δ or PPAR γ in retinas of mice. Moreover, down-regulation of PPAR α played a dominant role in the development of DR, while PPAR β/δ or PPAR γ showed limited influence^[20]. PPAR β/δ is mainly expressed in skeletal muscle and bone^[21]. PPAR γ is distributed mainly in adipose tissue and the colon. Also, PPAR γ expression is more than other PPARs in macrophages. PPAR γ can influence monocyte differentiation into M2 macrophages, while PPAR α or PPAR β/δ cannot^[22]. It is the same of the highest expression level of PPAR γ and its dominant regulatory role in cerebrospinal fluid^[23]. In the current study, we demonstrated the prominent expression of PPAR γ on the ocular surface and in the lacrimal gland, which suggested that PPAR γ may play the dominant regulatory role among PPARs in ocular surface and lacrimal diseases. Our results may help to direct further research into metabolic disorders on the ocular surface and in the lacrimal gland.

The fact that insulin and insulin-like growth factor-1 (IGF-1) receptors are present in human tear film and on the human ocular surface^[24], suggests that the dysregulation of glucose metabolism regulated by PPAR γ may contribute to the development of ocular surface diseases and the potential regulatory effect of PPAR γ on the ocular surface. Furthermore, through the analysis of the pathways related to PPAR γ via the DAVID and STRING database, using rat diabetic and dry eye models, our data demonstrated that the expression of PPAR γ is down-regulated in the lacrimal gland of both diabetic and dry eye rats. We also showed decreased expression of Sirt1, another major regulator of insulin sensitivity^[25], and metabolic and inflammatory pathways^[26-27]. Decreased expression of Sirt1 was observed in diabetic mice exposed to dry eye stimulation, and the mice developed seriously wounded corneal epithelia and decreased tear production^[28]. The negative regulation between PPAR γ and Sirt1 has been widely studied in the development of fibroblasts^[29], adipocytes^[30] and the placenta^[31]. The relationship between these two main factors is by no

means simple, and has rarely been reported for the ocular surface or the lacrimal gland. Although the cross-talk between PPAR γ and Sirt1 is complicated and controversial^[31], the down-regulation of the transcription of PPAR γ and Sirt1 reported in the current study suggests them as potential therapeutic targets for the treatment of diabetic-related dry eye disease. The underlying mechanism warrants further investigation.

Bioinformatics analysis focused on PPAR γ showed that PPAR γ was actively involved in the inhibition of multiple inflammatory-signaling pathways, rather than the glucose-metabolism pathway. Several pro-inflammatory genes, such as TNF- α , IL-6, IL-1 β , MyD88, and TGF- β are potentially directly or indirectly regulated by PPAR γ . For example, activation of PPAR γ facilitates its beneficial anti-inflammation function *via* the inhibition of the TLR4/MyD88 pathway *in vivo* and *in vitro*^[32-35]. The activation of PPAR γ promotes the expression of HIF-1 α ^[36], which may protect the lacrimal gland from inflammation^[37-38] or diabetes^[39-40] through down-regulation of the expression of IL-6, IFN- γ , VEGFA, TLR4, IFN- γ , and TNF. Furthermore, the cytokine-cytokine receptor interaction pathway involves IL-6, TNF, IFN- γ , and TGF- β . Up-regulation of PPAR γ inhibits the expression of TGF- β ^[41] and provides a protective effect against dry eye diseases^[42-44] and against organ fibrosis, including lacrimal fibrosis induced by diabetes^[42-47]. Indeed, our quantitative polymerase chain reaction (qPCR) data demonstrated that the expression of those pro-inflammatory cytokines were up-regulated on the ocular surface in both dry eye rats and diabetic rats, suggesting that diabetic corneal alteration and dry eye disease might share common inflammatory responses, which could be protected against by the activation of PPAR γ .

Animal models for dry eye studies have been developed through subcutaneous injection of scopolamine hydrobromide^[48], surgical resection of the main lacrimal gland^[49], instillation of benzalkonium chloride^[50] or maintenance in a dry environment. There are also spontaneous Sjögren's syndrome mice^[51]. Among these models, the low-humidity environment used in the current study is more relevant to human dry eye syndromes than are drug-induced models. Although the severity of dry eye disease due to low humidity is considerably milder than that induced by drugs^[52], the model still displays the obvious phenotype on the molecular biology level shown in the current study.

Lacrimal gland dysfunction, caused by desiccating-stress or diabetic conditions, not only shows similar signaling pathways but also displays common pathologic changes. Our data show that desiccating stress and diabetes can lead to a decrease in tear secretion and to corneal injury. The decrease of PPAR γ expression is highly to the similarly changes of relevant factors in these two groups. These findings may be used to explain

the exacerbated development of dry eye syndrome in diabetic populations, compared with non-diabetic patients, and provide insight regarding why PPAR γ may be a potential therapeutic target for lacrimal gland dysfunction related to dry eye diseases.

There are still many limitations in our experiments. It might have been better for us to measure the inflammatory cytokine levels in the tears of rats in the three groups. Furthermore, we should measure the inflammatory cytokine levels and osmolarity in the tears of patients with dry eye or diabetes. We will continue to focus our research on diabetes-associated dry eye.

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