·Basic Research·

Expression of peroxisome proliferator-activated receptorγ in rat retina during development

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Abstract

• AIM: To evaluate the spatiotemporal expression pattern of PPARγ in embryonic and early postnatal stages of rat retina.

• METHODS: Fetal rats were collected at 13-18d of gestation (GD) from pregnant females and postnatal rats at 1d (P1) and 5d (P5) after birth were also used. We used RT-PCR to detect PPARγ mRNA and immunohistochemical to observe PPARγ protein. And at last, we chose HE staining showed the structural changes of rat retina during development.

• RESULTS: RT-PCR analysis showed that PPARγ mRNA was expressed as early as GD13 and gradually decreased as maturation continued. However, the PPARγ gene expression significantly increased after birth, especially in P5. Immunohistochemical analysis showed PPARγ protein was expressed throughout the retinal neuroepithelium at GD13 and GD14, and then decreased during late embryogenesis but remained relatively high in the predicted ganglion cell zone. During postnatal development, PPARγ protein was remarkably increased and the positive signals were mainly located in nerve fiber layer (NFL), ganglion cell layer (GCL) and outer layers of the retina.

• CONCLUSION: The spatiotemporal changes of PPARγ expression demonstrated that PPARγ might play a role in regulating the differentiation and maturation of retinal cells.

• KEYWORDS: peroxisome proliferator-activated receptorγ; development; rat retina

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INTRODUCTION

Peroxisome proliferator-activated receptor γ (PPARγ) belongs to the nuclear hormone receptor superfamily and has three characterized, PPARγ1, γ2, and γ3. With ligand binding, PPARγ form heterodimers with the retinoic X receptor and bind to PPAR response elements in the promoter region of specific target genes, thus regulating their expression [1-3].These receptors have been found in teleosts, amphibians, rodents and humans [4]. Rat and mouse PPARγ1 and γ2 have 96.5% homology with the human receptors[5]. PPARγ is expressed most abundantly in adipose tissue, where it promotes dipocyte differentiation and regulates the expression of genes involved in fatty acid metabolism [6,7]. Many studies have shown a more widespread distribution of PPARγ receptors, suggesting that PPARγ ligands may have effects in other tissues. These actions include inhibition of inflammatory processes in macrophages, neointima formation after vascular balloon injury, growth of cancer cells, and neuroprotective effects[6,8-12]. PPARγ exerts various important functions for development and differentiation[13]. In central nervous system (CNS), PPARγ is widely expressed in neuronal stem cells (NSCs) in both embryo and adult mouse brains[14-15], and PPARγ activation by its agonists may influence neuronal differentiation [16-18]. Severe brain development disorders are observed in PPARγ-/- and PPARγ-/+ mice embryos [19]. Recently, Ghoochania *et al*[20] investigated the involvement of PPARγ in two stages of neural differentiation of mouse embryonic stem cells, during and post-neural precursors formation. They found PPARγ inactivation during neural precursors formation reduced expression of neural precursor and neural (neuron and astrocyte) markers. However, PPARγ inactivation at post-neural precursors formation stage only decreased the expression of mature astrocyte marker. These results demonstrated the importance of PPARγ in neural differentiation.

The eyeball, especially retina, is the extended part of the brain, therefore, eye development is highly correlated with the CNS. PPARγ has been provided express in retina, and participate in many physiological and pathological processes, such as diabetic nephropathy [21,22], age-related macular degeneration[23,24], and retinal neuroprotection[25-27]. However, the expression of PPARγ in rat retina during development has not been described. In the present study, we investigated

the change of PPARγ in embryonic and early postnatal stages of rat retina using reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry and explore the role of PPARγ in retinal development.

MATERIALS AND METHODS

Animals Adult Sprague Dawley rats of both sexes, weighing 200-300 g were obtained from the Animal Experimental Center of Nantong University, in line with the National Medical Standards for animal use of clean grade. All animals were fed with standard diet and water, and were housed in a climate-controlled room with a 12L:12D schedule. Two females were paired with a male overnight and the next morning, males were removed and females were assessed for the presence of sperm in the vaginal flush. Animals with positive sperm in the flushes were designated as day 1 of gestation (GD1). Fetal rats were collected at 13-18d of gestation from pregnant females and postnatal rats at 1d (P1) and 5d (P5) after birth were also used. At each time point, 6 rats were used for mRNA analysis and 6 rats were used for histology and immunohistochemistry staining. All procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996, USA) and were approved by the Jiangsu Branch of Chinese National Committee to the Use of Experimental Animals for Medical Purposes.

Reverse Transcription-polymerase Chain Reaction PPARγ mRNA was detected by RT-PCR and total RNA was extracted by Trizol reagent. Absorbance A260 and A280 of RNA were measured by violet spectrophotometry to calculate concentration of total RNA sample. cDNA was synthesized based on the introduction. Primer was synthesized in the Bioengineering Technology Co., Ltd., Shanghai (Table 1). All products were optimized for both cycle number and product amplification using β-actin as a control for equal loading. The PCR mixture contained reaction mix, RT enzyme mix, and DEPC water and incubated at 25℃ for 10min. Then incubated for 30min at 50℃, the reaction was terminated at 85℃ for 5min, and the mixture was chilled on ice. One microliter (2 U) of Escherichia coli RNase H was added to the reaction and incubated at 37℃ for 20min. The cDNA was then stored at -20℃. Relative mRNA concentrations were calculated from take off point of reactions using the software provided by the manu-facturer, and normalized to β-actin expression level.

Histologic and Immunohistochemical Analysis At each time point, the rats were sacrificed and the eyeballs were harvested. The eyeballs were washed with 0.01 mol phosphate buffer saline (PBS) and fixed in 4% of formaldehyde solution for 24h at 4℃. Frozen sections were made with a thickness of 6 μm. The sections were then stained with haematoxylin and eosin (HE) and observed with a light microscope (Leica, Germany). For immunohistochemical analysis, nonspecific binding sites were blocked for 1h with PBS containing 2% donkey serum, 2% bovine serum albumin (BSA) and 0.2% TritonX-100. Sections were incubated with the antibody against PPARγ (Santa Cruz, 1:50) overnight at 4℃. Polink-2 plus Polymer HRP Detection System For Goat Primary Antibody (GBI Co., USA) was used to detect the goat IgG. The HRP activity was revealed by diaminobenzidine (DAB). The slices were counterstained with hematoxylin and then examined on a Leica light microscope (Germany).

Table 1 The primers used were as follow

Gene		Primer sequence
PPARγ	sense	tgg agc cta agt ttg agt ttg c
	antisense	tga ggt ctg tca tct tct gga g
β-actin	sense	cac ccg cga gta caa cct tc
	antisense	ccc ata ccc acc atc aca cc

Statistical Analysis Data were expressed as Mean±SEM. One way ANOVA was used for assessing the differences in PPARγ mRNA expression during different days of gestation. Values were considered significant at $P<0.05$.

RESULTS

Expression of PPARγ mRNA in the Developing Rat Retina In order to clarify the expression trend of PPARγ during retinal development, we extracted RNA from the whole retina of GD13 to GD18 fetal rats, and from P1 and P5 newborn rats, and analyzed by RT-PCR technique. The results showed that PPARγ mRNA was expressed as early as embryonic stage 13. As maturation continued, the expression of PPARγ mRNA gradually decreased. We found a very low level of PPARγ mRNA in GD18, however, the PPARγ gene expression significantly increased after birth, especially in P5 (Figure 1).

Expression and Localization of PPARγ Protein in the Developing Rat Retina Similarly to other rodent, the SD rat retina is undeveloped at birth. The retina was composed of two layers at GD13: assumed retinal pigment epithelium (RPE) and assumed neuroepithelium, but the boundary was not clear and the cells were irregular arranged. As time goes by, the boundary became increasingly clear and ganglion cell layer (GCL) gradually appeared in the innermost layer of neuroepithelium. At GD18, chievitz layer was detected (Figure 2). PPARγ immunolabeling signals were observed throughout the retinal neuroepithelium at GD13 and even stronger at GD14. From GD15 to GD18, a dramatic decline in the expression of PPARγ was occurred. However, the expression of PPARγ was remarkably increased after birth. At P1, PPARγ positive cells were distributed mainly in the nerve fiber layer (NFL), GCL and also observed in a scattered pattern in inner plexiform layer (IPL). At P5, there was strong staining of PPARγ in the retinal NFL, GCL and outer layers as well as throughout the retina (Figure 3).

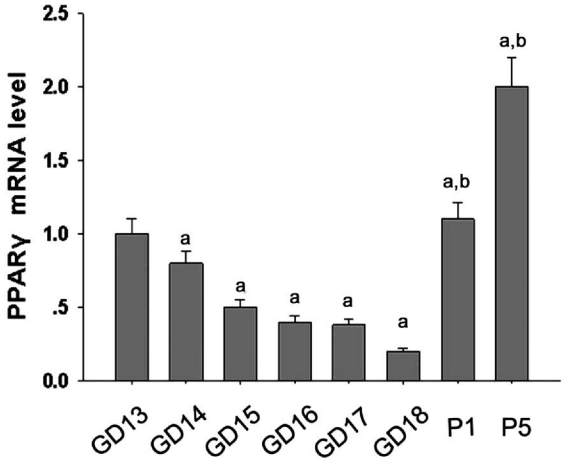

Figure 1 The expression of PPARγ mRNA in rat retina during embryogenesis Total RNA was obtained from each SD rat embryo development stages. RT-PCR was performed at least three times and the expression of PPARγ was normalized by β-actin. [a]Significantly different compared with GD13; [b]Significantly different compared with GD18. $P<0.05$.

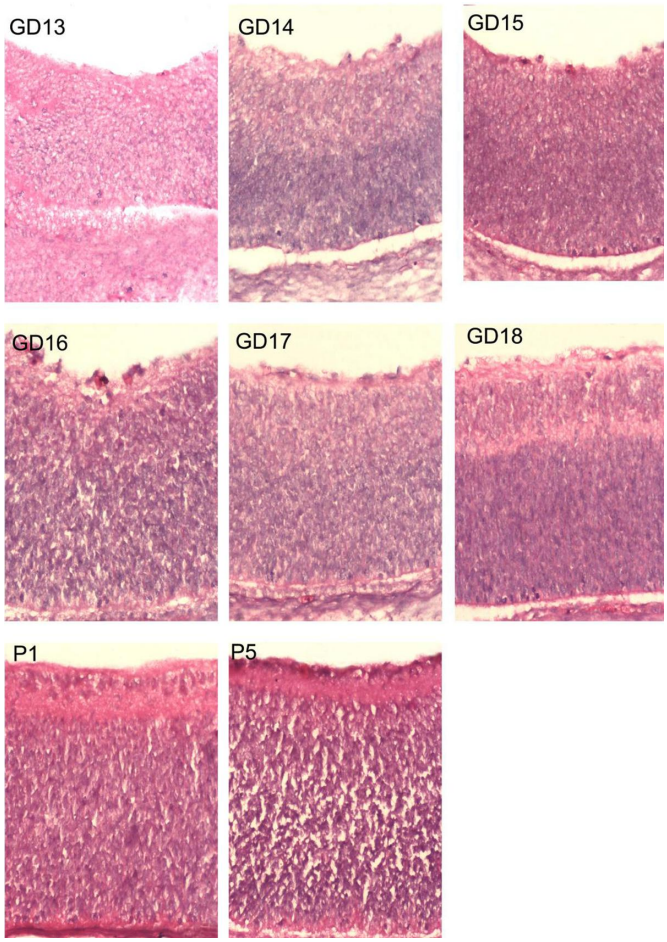

Figure 2 Structural changes of retina during embryogenesis The HE staining of retina from GD13 to 18 , P1 and P5, adult. The SD rat retina is undeveloped at birth. At GD15, the retina was composed by two layer: RPE and NBL. At GD18, chievitz layer was appeared. At P5, INL and ONL tended to become separate.

DISCUSSION

Based on the physiological roles of PPARγ, it is expected to have important roles in differentiation and development.

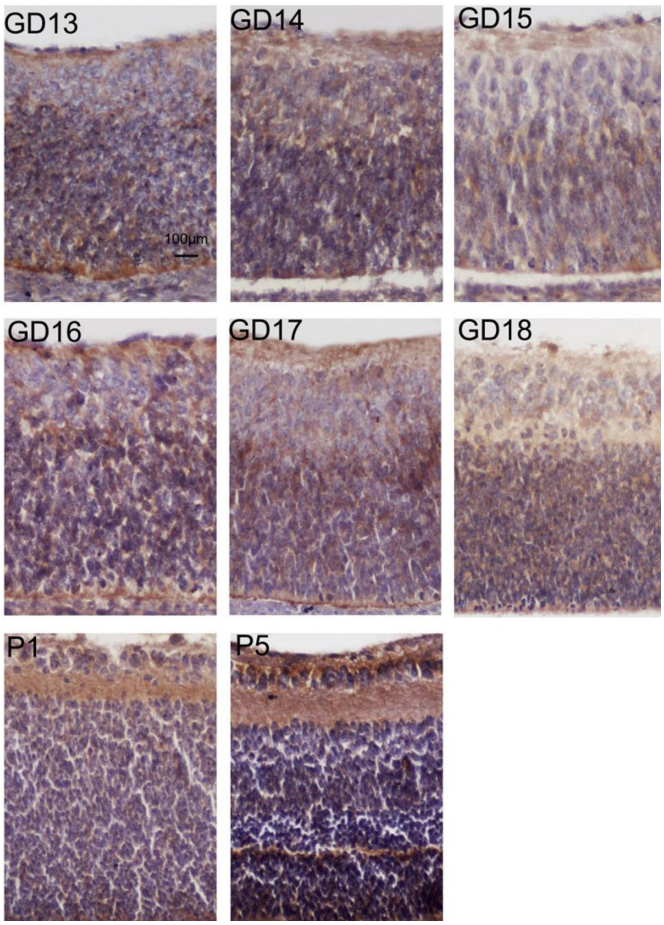

Figure 3 The expression of PPARγ protein in rat retina during embryogenesis Immunohistochemical analysis of retina from GD13 to 18, P1 and P5, adult. Positive staining was found in the outer of NBL in retina at GD13, nearly no positive at GD18, in P5 it was observed in NFL, IFL and the outer of NBL, and in adult was mainly in the NFL and INL.

Studies on rat and human embryos have established that PPARγ is expressed in the rodent and human embryo at early developmental stages, mainly in the tissues where they will be found postnatally and in the adult. The presence of PPARγ is faintly detected in embryonic cell nuclei of mouse as soon as day-5 post-fecundation. At midgestation PPARγ is highly expressed in the hindbrain, the spinal cord, the vertebrae, the heart and the brown adipose tissue [28]. In the present study, we described the expression of PPARγ during retinal development. This is the first study, to our knowledge, that has examined expression of this gene in the embryonic and neonatal rat retina. At GD13, we detected a relatively high expression of PPARγ mRNA and protein. As the embryo developed, the expression of PPARγ gradually decreased and at GD18, only a weak expression was observed. However, stronger expression levels were appeared at early postnatal stage. Similar results [28,29] were found in the rat CNS embryogenesis. Braissant and Wahli[29] reported that PPARγ was expressed at moderate levels on GD13.5 in the rat brain, decreased by GD15.5 and was not

detected on GD18.5, and it was weakly found in adult rat brain.

In order to investigated the localization of PPARγ, we performed immunohistochemistry and HE staining. Consistent with previous report [30,31], we found the retinal histological structure and cellular morphology of embryonic rat were considerably different from those of adult rats. The retina of SD rat differentiated from embryo stage but still immature at birth. The GCL has separated from the inner layer at P1, but the inner nuclear layer (INL) and outer nuclear layer (ONL) have not become separated, and INL and ONL began to separate from P5. PPARγ was expressed through all the neuroretinal layers at GD13. Form GD15 to 18, the levels of PPARγ was decreased but remained relatively high in the predicted ganglion cell zone. During postnatal development, the positive signal was found at all retinal layers at P5. The distribution of PPARγ in the developing retina suggested that it may play a role in regulating the differentiation and maturation of both retinal neurons and glial cells.

The exact function of PPARγ in retinal development is not clear. It has various roles in cell proliferation and differentiation in neuroblastoma cells GD13.5 corresponds to the onset of differentiation and apoptosis events in CNS[15, 29]. As a member of CNS, the transient peak of expression in retina would suggest a role for PPARγ during these processes. The expression levels of PPARγ were increased to maximum in the neonatal rat eye retina. This is consistent with the fact that after the birth period, active rat retinal differentiation occurs while retina maturation does not occur until several weeks after birth.

In conclusion, our expression analysis of the embryonic and neonatal rat showed that PPARγ was expressed by GD13 and than decreased during late embryogenesis, but its expression was up-regulated at postnatal maturation. The spatiotemporal changes of PPARγ expression demonstrated that PPARγ might play a role in regulating the differentiation and maturation of retinal cells. However, it is still unclear that whether PPARγ expression starts the transformation, what other methods can induce PPARγ expression, and which downstream gene transcriptions started by PPARγ activation may take part in embryonic development of retina. Therefore, significance and mechanism of PPARγ in retinal development still need further studies.

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