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Rosiglitazone ameliorates senescence-like phenotypes in a cellular photoaging model

Liang Chen [a,1], Bo Bi [b,1], Jiping Zeng [a], Yiqun Zhou [a], Ping Yang [a], Yu Guo [a], Jingjing Zhu [a], Qingjian Yang [a], Ningwen Zhu [c], Tianyi Liu [a,*]

[a] Department of Plastic and Reconstructive Surgery, Hua Dong Hospital, Fu Dan University, Shanghai, China
[b] Department of Laboratory Medicine, Hua Dong Hospital, Fu Dan University, Shanghai, China
[c] Department of Dermatology, Hua Shan Hospital, Fu Dan University, Shanghai, China

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ABSTRACT

Background: Rosiglitazone (RO), a second-generation thiazolidinedione used mainly in the treatment of non-insulin-dependent diabetes mellitus, has been discovered to be a high-affinity ligand for peroxisome proliferator-activated receptor-γ (PPAR-γ). Several studies have revealed that PPAR-γ is also involved in the regulation of oxidative stress and chronic inflammation associated with aging process in vivo as well as with cellular senescence in vitro. We sought to investigate whether RO pretreatment will counteract the photoaging process using a well-established cellular photoaging model.

Methods: Murine dermal fibroblasts (MDFs) were cultured in the absence or presence of RO for 48 h, followed by exposure to repeated UVB irradiation. The senescent phenotypes were evaluated including cell viability, senescence-associated β-galactosidase (SA-β-gal) expression, cell morphology, ROS generation, cell cycle, production and degradation of extracellular matrix (ECM), and the potential mechanisms were discussed.

Results: Pretreatment with RO (40 μM) significantly decreased the staining intensity and the percentage of SA-β-gal-positive cells and reserved the elongated cell shape compared with UVB group. The cells pretreated with RO also showed decreased UVB-induced degradation of type I collagen by decreasing MMPs expressions. In addition, we observed counteraction of cell-cycle arrest and repression of UVB-induced p53 and p21 in the presence of RO. We further confirmed a significant decrease in ROS accumulation accompanied by an increase in catalase in RO group.

Conclusions: RO, a potent PPAR-γ activator, counteracts senescence-like phenotypes, including long-term growth arrest, flattened morphology, degradation of ECM and SA-β-gal-positive staining in MDFs by inhibiting the expression of MMPs and increasing the synthesis of catalase when administered to repeated UVB irradiation. The novel application of RO may lead to innovative and effective anti-photoaging therapies.

1. Introduction

Ultraviolet radiation, which is the main factor causing changes associated with the aging process in sun-exposed areas, results in extrinsic skin aging, also referred to as photoaging [1]. The dermal fibroblasts, which suffer accumulated damage during ultraviolet irradiation, play a key role in photoaging due to their important role in maintaining the metabolic balance of dermal extracellular matrix (ECM) [2]. The accumulation of reactive oxygen species (ROS) and the increase of matrix metalloproteinases (MMPs) induced by ultraviolet irradiation in fibroblasts reflect the central aspects of photoaging process [1,3]. Given the important roles of ROS and MMPs, one priority is given to identify some compounds that are capable of promoting antioxidant defense and reducing MMPs, offering future perspectives for skin photoprotection.

Rosiglitazone (RO), a known potent synthetic ligand of peroxisome proliferator-activated receptor-γ (PPAR-γ), effectively increases glucose uptake and decreases insulin resistance by activating a number of genes in tissues through PPAR-γ, which belongs to a family of nuclear hormone receptors that regulate the function and expression of complex gene networks, especially

* Corresponding author.
E-mail address: tianyiliucn@126.com (T. Liu).
[1] These authors contributed equally to this work.

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involved in cell proliferation and differentiation, glucose metabolism and homeostasis, insulin sensitivity and lipid metabolism [4–6]. Currently, RO is one of the most commonly prescribed insulin sensitizers used in patients with type 2 diabetes mellitus in the world. Although the widespread use of RO is attributable to its regulation of lipid metabolism and glucose homeostasis, PPAR-γ possesses numerous other effects by means of regulating the function and expression of complex gene networks independent of its antidiabetic capability. Several recent studies have revealed that PPAR-γ inhibits the expression of MMPs and acute-phase proteins [7]. In addition, PPAR-γ also modulates oxidative stress-sensitive pathways and the expression of AP-1 [8]. Taken together, these studies suggest its potential as therapeutic target for photoaging, a process that has been generally ascribed to accumulated oxidative insults.

Given the significant role PPAR-γ plays in conjunction with the well-established activation of PPAR-γ seen with RO in vivo, the purpose of our study was to investigate the potential effect of RO pretreatment on photoaging process in vitro. We have previously developed a reproducible photo-damaged model of mouse dermal fibroblasts (MDFs) by repeated UVB exposures, which demonstrates a series of senescence-like phenotypes, including long-term growth arrest, flattened morphology, increased synthesis of MMPs, increased degradation of ECM and senescence-associated-β-galactosidase (SA-β-gal) staining [9]. We hypothesized that RO pretreatment for MDFs would counteract the process of senescence induced by repeated UVB exposures through the activation of PPAR-γ. In addition, we attempted to elucidate the possible molecular mechanisms underlying its inhibitory effects.

2. Materials and methods

2.1. Cell culture and treatments

All animal experiments were performed with the approval of the Animal Care and Use Committee of Fudan University. MDFs were isolated from the dorsal dermis of newborn littermates of C57 BL/6 mice 18–24 h after birth, as previously described [10]. The cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS), glutamine, penicillin and streptomycin in a 37 °C humidified incubator containing 5% CO_2. The fibroblasts were cultured to 80% confluence and then subcultured. Cells of passage 1–2 were used for the following experiments.

At 12 h after plating, cells were stimulated with RO in DMEM with 10% FBS for 48 h. Then supernatant was replaced with a thin layer of phosphate-buffered saline (PBS) and cells were exposed to a series of 4 doses of UVB at 120 mJ/cm^2 (Narrowband TL 20 W/01 RS lamp, Philips, The Netherlands) for 120 s with an interval time of 12 h as previously described [9]. Immediately after each irradiation, the PBS was aspirated and replaced with DMEM containing RO in 1% FBS. Control cells were kept in the same culture conditions without UVB exposure and RO treatment. After a series of 4 times of exposure, the cells were allowed for recovery in DMEM with 10% FBS.

2.2. Cell viability assays

Cell viability of MDFs exposed to RO (0–80 μM) up to 96 h was determined using WST-8 dye (Cell Counting Kit-8, Beyotime Inst Biotech, China) according to manufacturer's instructions. Briefly, 5×10^3 cells/well were seeded in a 96-well flat-bottomed plate. After 12 h, the medium was replaced with DMEM containing RO of increasing doses and then cultured in a 37 °C humidified incubator containing 5% CO_2 for another 48 and 96 h. After incubation, 10 μl of WST-8 dye was add to each well, cells were incubated at 37 °C for 2 h and the absorbance was finally determined at 450 nm using Varioskan Flash Spectral Scanning Multimode Reader (Thermo Electron Corporation, USA). The modification in viability of in RO-treated fibroblasts was expressed as a percentage compared to nontreated cells and the control cells were considered to be 100% viable.

2.3. SA-β-Gal staining

SA-β-Gal staining was performed as previously described [11]. The staining kit was purchased from cell signaling technology (#9860, Boston, MA, USA). At 24 h after the last UVB treatment, cells were seeded in 35 mm dishes and cultivated for another 48 h in DMEM + 10% FBS. The cells were washed in PBS, fixed for 10 min (room temperature) in 4% paraformaldehyde and stained according to manufacturer's instruction. The population of SA β-Gal-positive cells was determined by counting 400 cells per dish using Image-Pro Plus (IPP) 6.0 software (Media Cybernetics, Bethesda, MD, USA). The proportions of cells positive for the SA β-Gal activity were given as percentage of the total number of cells counted in each dish.

2.4. Fluorescent labeling of cytoskeletal proteins

At 24 h after the last UVB treatment, cells were seeded in 35 mm dishes and cultivated for another 48 h in DMEM + 10% FBS. The cells were washed in PBS, fixed in 4% paraformaldehyde, permeabilized in 0.3% Triton X-100 for 15 min under room temperature, and then incubated with FITC-Phalloidin (Sigma Chemical Co., USA) for 30 min. Nuclei were identified with 4′,6-diamidino-2-phenylindole (DAPI). Images were recorded at 200× using a fluorescent microscope (Olympus IX70-S1F2, Japan).

2.5. Flow cytometry analysis for cell cycle

Cell-cycle analysis was performed by flow cytometry at 48 h after the last UVB treatment. Briefly, cultured cells were trypsinized into single-cell suspensions and fixed with 70% ethanol overnight at 4 °C. RNA was degraded by incubation with 1 mg/ml RNase (Sigma-Aldrich, St. Louis, MO, USA) for 1 h at 37 °C. DNA was labeled with 50 μg/ml propidium iodide (PI; Sigma-Aldrich) and DNA content was assessed by Beckman Coulter Epics Altra flow cytometer (Beckman Coulter, High Wycombe, UK) equipped with the ModiFit LT v2.0 software.

2.6. RNA extraction and real-time RT-PCR

At 48 h after the last irradiation, total cellular RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA). cDNA was synthesized from 2 mg total RNA using 200 U of reverse transcriptase (MMLV RT) and 20 pM oligodT (Promega, Madison, WI). The primers were listed in Table 1.

Amplification reactions assays contained 1× SYBR Green PCR Mastermix and primers (Applied Biosystems, The Netherlands) at optimal concentration. A hot start at 95 °C for 10 min was followed by 40 cycles at 95 °C for 30 s, 60 °C for 30 s and 72 °C for 45 s using the StrataGene Mx3000p (Agilent Technologies, USA). Melting curves were generated after amplification and data were analyzed using the Mxp software. Each sample was tested in triplicate and three independent repeating experiments were performed.

2.7. Protein quantification and western blotting

For quantification of soluble protein, cells were harvested at 72 h after the last UVB irradiation. The cell number was counted in each group and cells were lysed for quantification of protein using BCA kit (Pierce, USA). The amount of soluble protein was divided by cell numbers as a marker of cellular hypertrophy (protein/cell).