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Research Paper

Establishment of a cell-based drug screening model for identifying agonists of human peroxisome proliferator-activated receptor gamma (PPARγ)

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Keywords
drug screening; HEK293T cells; peroxisome proliferator-activated receptor gamma; reporter gene; transfection

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Abstract

Objectives Peroxisome proliferator-activated receptor gamma (PPARγ) plays a critical role in regulation of diverse biological processes, including lipid metabolism and adipogenesis, cell division and apoptosis, and is involved in variety of disease conditions, such as obesity, atherosclerosis, inflammation and tumour. Developing a cell-based reporter gene model targeting PPARγ would be useful to screen human PPARγ agonists that could be beneficial to patients with these diseases.

Methods We stably co-transfected human embryonic kidney (HEK) cell line 293T cells with phPPARγ-IRES2-EGFP vector to express human PPARγ (hPPARγ), a reporter vector pPPRE×3-TK-LUC, and control vector pRL-CMV. The efficiency of the co-transfection was evaluated with flow cytometry of hPPARγ expressing cells. Specificity of hPPARγ activity was determined by dual luciferase reporter assay of co-transfected cells exposed to PPARγ agonist rosiglitazone, PPARα agonist WY14643 and retinoic acid receptor alpha (RARα) agonist all-trans-retinoic acid (ATRA).

Key findings The phPPARγ-IRES2-EGFP co-transfected HEK293T cells showed concentration- and time-dependent luciferase induction upon exposure to the rosiglitazone, while WY14643 and ATRA were unable to activate the co-transfected HEK293T cells.

Conclusions These data indicated that the HEK293T cells could be stably transfected with hPPARγ. This cell-based drug screening platform could be used targeting specific nuclear receptor of hPPARγ with effectiveness and specificity for hPPARγ agonists discovery.

Introduction

Peroxisome proliferator-activated receptor gamma (PPARγ) is a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors, and plays a critical role in a variety of biological functions including regulation of lipid metabolism, inflammation, cellular differentiation, and apoptosis.[1–5] Ligand-activated PPARγ acts as a transcription factor and forms a heterodimer complex with retinoic acid receptor alpha (RARα). It can then bind to the peroxisome proliferator responsive element (PPRE) within the promoter of target genes and regulate their expression.[6] Two isoforms of PPARγ have been reported, PPARγ1 and PPARγ2, which are generated by alternative promoter usage. Compared with PPARγ2, which is predominantly expressed in adipose tissue, PPARγ1 is widely expressed in body tissues.[7]

Recent studies have reported that activation of PPARγ by ligands is associated with beneficial health effects including antiobesity, antihypertension, anti-atherosclerosis, anti-diabetes, anticancer and other diseases.[8–10] Those studies implied that PPARγ might become a possible new target for therapy for the above disorders. To date, the only available high-affinity drugs targeting PPARγ are the thiazolidinediones, a class of antidiabetic drugs, which are widely prescribed in clinic for improving insulin sensitivity in type 2 diabetes.[11–13] Nevertheless, most research has shown that prescription of thiazolidinediones could increase the incidence of myocardial infarction, stroke, heart failure, and even death from cardiovascular diseases.[14–17] Finding new PPARγ agonist drugs with less side effects is needed.

Therefore, we have attempted to establish a cell-based human PPARγ (hPPARγ) drug screening platform, which could be used in screening new drugs with PPARγ activating function. In this study, we chose human embryonic kidney (HEK) 293T cells as a biological carrier and imitated the hPPARγ signalling pathway through co-transfection of recombinant plasmid phPPARγ-IRES2-EGFP, firefly luciferase-containing report plasmid pPPRE×3-TK-LUC and renilla luciferase-containing internal control plasmid pRL-CMV into HEK293T cells. Thereafter, we chose the PPARγ specific agonist rosiglitazone as a positive drug to evoke this artificial hPPARγ signalling pathway model. The reporter gene assays suggested that rosiglitazone could enhance the transcriptional activity of hPPARγ with a dose-dependent and time-dependent manner in the cell model. Moreover, the PPARα agonist WY14643 and RARα agonist all-trans-retinoic acid (ATRA) could not activate the model. The results suggested that an effective and specific cell-based screening model for human PPARγ ligand agonists was established here successfully, and could be used in future screening for candidates of unknown hPPARγ ligands or activators.

Materials and Methods

Cells and cell culture

Human embryonic kidney cells HEK293T were obtained from the Chinese Academy of Sciences Shanghai Cell Bank. The cells were maintained in Dulbecco's modified Eagle's medium with high glucose (HG-DMEM) (Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum, 100 U/ml benzylpenicillin (penicillin G), 100 μg/ml streptomycin, and 4 mmol/l L-glutamine (Hyclone, Logan, UT, USA), at 37°C in 5% CO_2 humidified incubator.

Plasmids amplification and extraction

Recombinant plasmid phPPARγ-IRES2-EGFP was constructed as described previously.[18] phPPARγ-IRES2-EGFP and empty vector pIRES2-EGFP in *Escherichia coli* were cultured in Luria-Bertani (LB) medium containing 30 μg/ml kanamycin. Reporter plasmid pPPRE×3-TK-LUC (a gift from Dr Ronald M. Evans, Salk Institute for Biological Studies, San Diego, CA, USA) and internal control plasmid pRL-CMV were cultured in LB medium containing 50 μg/ml ampicillin.[19] After culture in a constant temperature oscillation incubator at 37°C, approximately 160–180 rev/min, for approximately 10–12 h, plasmids were extracted and purified with Wizard Plus SV Minipreps DNA Purification System (Promega, Madison, WI, USA) according to the manufacturer's instructions.

Plasmids co-transfection

HEK293T cells were harvested from an actively growing culture and plated into Costar 24-well plates (Corning, Cambridge, MA, USA) at 8×10^4 cells/well in 500 μl HG-DMEM without antibiotics. After 24 h at 90% confluence, they were transfected with a mixture of the Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) and plasmid DNA according to the manufacturer's instructions. Briefly, phPPARγ-IRES2-EGFP (0.5 μg), reporter plasmid pPPRE×3-TK-LUC (0.25 μg) or internal control plasmid pRL-CMV (0.005 μg) were diluted into 50 μl HG-DMEM without serum and antibiotics. Lipofectamine 2000 was diluted at the appropriate amount of 2 μl Lipofectamine 2000 in 50 μl HG-DMEM without serum and antibiotics, and incubated for 5 min at room temperature. Diluted Lipofectamine 2000 was added to the diluted plasmids DNA mix at a lipid (μl) : DNA (μg) ratio of 2.5 : 1, and incubated for 20 min at room temperature. The plasmid-lipid mixture (total volume of 100 μl) was added to each well, and incubated at 37°C in a water-saturated atmosphere with 95% ambient air and 5% CO_2. Meanwhile, the empty vector pIRES2-EGFP, reporter plasmid and internal control plasmid were co-transfected into HEK293T cells as described above. The efficiency of transfection was evaluated by flow cytometry (FACS caliber, Becton Dickinson, San Josè, CA, USA) and inverted fluorescence microscopy (DMIRB DIC, Leica, Germany) at 36 h post-transfection.

Drug intervention and dual luciferase reporter assay

Fourteen hours after transfection of plasmids into HEK293T cells, the cell culture medium was replaced with complete media containing either indicated vehicle (0.1% dimethyl sulfoxide (DMSO)) or PPARγ agonist rosiglitazone (Cayman Chemical Company, Ann Arbor, MI, USA) at 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} or 10^{-8} mol/l. Luciferase activity was quantified 24 h later using the luciferase assay kit (Promega) according to manufacturer's instructions, and the ratio of firefly to renilla luciferase activity (F/*R*-values) in each sample was calculated.[20] To examine the time-dependent effects of rosiglitazone, co-transfected HEK293T cells were cultured with 10^{-5} mol/l rosiglitazone for 8, 16, 24, 36 and 48 h. At different time points, the luciferase activity was measured, and each sample's F/*R*-value was determined.

To determine the specificity of the cellular response, we replaced rosiglitazone with WY14643 (Sigma, St Louis, MO, USA), a specific agonist of PPARα, or ATRA (Sigma), a specific agonist of RXRα, followed by determining luciferase activity and F/*R*-values.

RNA preparation, reverse transcription and real-time PCR

After culture with different doses of rosiglitazone for 24 h, total RNA was extracted from HEK293T cells using TRIzol Reagent (Invitrogen) and 500 ng total RNA were used for reverse-transcription with PrimeScript RT Reagent Kit

Table 1 PCR primers for hPPARγ and hRXRα

Genes	GeneBank ID		Sequences	Size (bp)
hPPARγ	NM_005037.5	Forward	5′-AGGAGCAGAGCAAAGAGG-3′	474
		Reverse	5′-AGGACTCAGGGTGGTTCA-3′	
hRXRα	NM_002957.4	Forward	5′-AATGGCGTCCTCAAGGT-3′	176
		Reverse	5′-CGGCAGGTGTAGGTCAG-3′	
hGAPDH	NM_002046.3	Forward	5′-CCCCTTCATTGACCTCAACTACAT-3′	421
		Reverse	5′-CATGAGTCCTTCCACGATACCAA-3′	

hGAPDH, human glyceraldehyde-3-phosphate dehydrogenase; hPPARγ, human peroxisome proliferator-activated receptor gamma; hRXRα, human retinoid X receptor alpha.

(Takara Biotechnology, Co., Dalian, China) according to the manufacturer's instructions. PCR primers (Table 1) were designed by Primer Premier 5 software package and synthesized by Takara Biotechnology, Co. (Dalian, China) cDNA were amplified in a thermal cycler (iQ5 System, Bio-Rad, Hercules, CA, USA) in a 50 μl reaction mixture containing 25 μl SYBR Premix Ex Taq II (Takara) (2×) and 2 μl of each forward and reverse PCR primers (10 μM) and 4 μl (equivalent to 200 ng total RNA) of the template cDNA and 17 μl RNase-free dH_2O. For hPPARγ amplification, the cycling conditions were as follow: one denaturing cycle at 95°C for 30 s, followed by 50 cycles of 95°C for 5 s, 55°C for 30 s, and 72°C for 30 s. For hRXRα amplification, the cycling conditions were as follows: one denaturing cycle at 95°C for 30 s, followed by 50 cycles of 95°C for 5 s, 62°C for 30 s. hGAPDH was amplified in parallel and the products were used for normalization of hPPARγ products as described previously.[21] Each sample was added in triplicate. The size of PCR product was confirmed by electrophoresis on a 1% agarose gel stained with GoldView (SBS Genetech, Beijing, China). Purity of the amplified PCR products was determined by melting point analysis using the iQ5 software (Bio-Rad).

Statistical analyses

All experiments were conducted in triplicate and the data were presented as mean ± standard error (SEM). Statistical analyses were performed using the SPSS 13.0 software package. Curve fitting and linear regression analysis was preformed to evaluate luciferase activity (F/*R*-values). The results were compared by the Mann-Whitney *U*-test. All values are reported as means ± SE. Statistical significance was set at $P < 0.05$.

Results

Expression of green fluorescent protein (GFP) and transfection efficiency

After transfection for 36 h, the adherent and irregular polygon shapes of HEK293T cells were observed by an inverted phase contrast microscope (Figure 1b), and a higher proportion and intensity of GFP positive HEK293T cells were observed under a fluorescence microscope in the same view (Figure 1a). Thereafter, the adherent cells were detached with 0.25% trypsin and the transfection efficiency of phPPARγ-IRES2-EGFP was measured by flow cytometry. The result showed that the transfection efficiency of phPPARγ-IRES2-EGFP in co-transfected HEK293T cells was 67% (Figure 1c).

Effects of rosiglitazone on the expression of luciferase

After culture of HEK293T cells with different doses of PPARγ agonist rosiglitazone for 24 h, a double-luciferase assay was carried out to determine the firefly luciferase luminescence values (F) and internal control renilla luciferase luminescence values (*R*) in each sample. The ratios of firefly to renilla luciferase luminescence (F/*R*-value) were used to normalize luciferase activity values. As shown in Figure 2a, rosiglitazone activated PPARγ-mediated luciferase reporter gene's transcription activity in a dose-dependent manner and the maximal efficacy was achieved at the dose of 10^{-6} M.

Curve fitting and regression analysis revealed a trend line that could be fitted to a cubic curve ($F = 51.777$, $P < 0.001$), and the regression equation with $y = 5.313 + 1,786x + 2.930x^2 - 0.456x^3$, and $R^2 = 0.951$. In addition, linear regression analysis showed a good linear relationship in the range of 0.1% DMSO~10^{-6} mol/l rosiglitazone, the regression equation was $y = 2.293 + 7.314x$, $r = 0.984$ ($t = 13.444$, $P < 0.001$). Compared with recombinant plasmid co-transfection group, F/*R*-values of the empty vector co-transfected group showed a low, close to baseline response by HEK293T cells cultured with different doses of of rosiglitazone.

To analyse the time course of rosiglitazone effects on PPARγ activity, rosiglitazone at 10^{-5} mol/l was added into co-transfected HEK293T cells, and the F/*R*-values were measured by double-luciferase assay at 8, 16, 24, 36 and 48 h. The results showed that the F/*R*-values increased in the HEK293T cells in a time dependent manner (Figure 2d). Curve fitting and regression analysis revealed that the trend line could be fitted to a

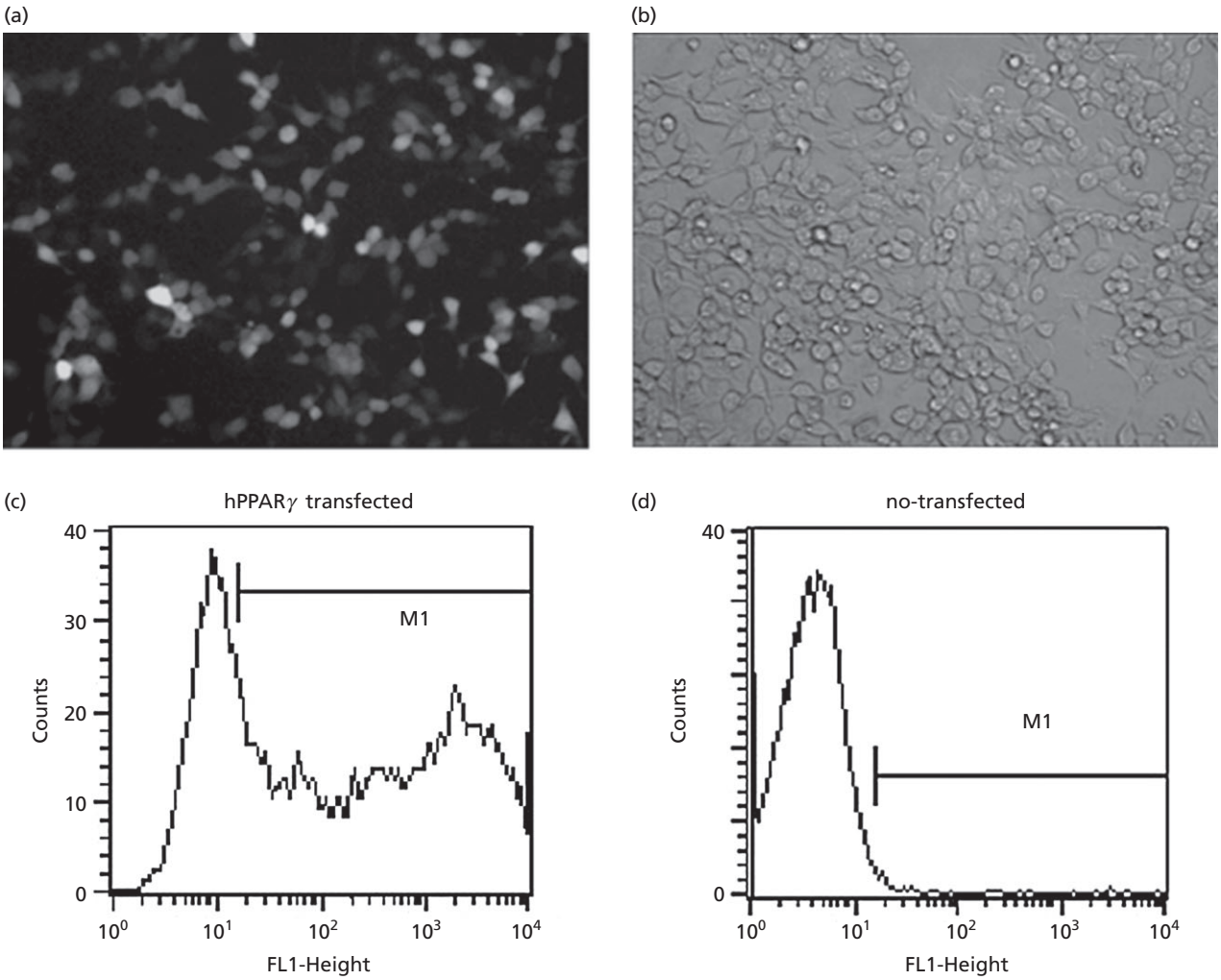

Figure 1 Transfection efficacy of phPPARγ-IRES2-EGFP in co-transfected 293T cells. (a) HEK293T cells were co-transfected with phPPARγ-IRES2-EGFP, pPPRE×3-TK-LUC, and pRL-CMV as indicated in 'Plasmids co-transfection'. Thirty-six hours after transfection, the expressions of GFP in HEK293T cells were observed under inverted fluorescence microscope. Original magnification ×200. (b) The same view of (a) under inverted phase contrast microscope. (c) Cells were then harvested, and the transfection efficacy of phPPARγ-IRES2-EGFP was measured by flow cytometry. (d) No-transfected HEK293T cells as control by flow cytometry.

cubic curve ($F = 88.691$, $P < 0.001$) when the regression equation was $y = -3.153 + 10.146\,X - 3.709x^2 + 0.455x^3$, $R^2 = 0.978$.

Notably, when the PPARγ agonist rosiglitazone was replaced with the PPARα agonist WY14643, the F/*R*-values were low and stable in the HEK293T cell culture (Figure 2b), indicating that the co-transfected cell model responded specifically to the PPARγ agonist, and could not be activated by PPARα specific agonist WY14643.

To determine whether the cell model could be activated through RXRα, the co-transfected HEK293T cells were cultured with ATRA, an RXRα-specific agonist. The F/*R*-values of ATRA-treated HEK293T cells were similar with that of WY14643-treated cells, indicating that the cell model could not be activated by ATRA through activation of RXRα (Figure 2c).

Expression of hPPARγ and hRXRα

Total RNA was extracted from HEK293T cells after culture with different doses of rosiglitazone for 24 h. Quantitative real-time PCR analysis revealed that the hPPARγ mRNA expression levels in phPPARγ-IRES2-EGFP co-transfected HEK293T cells were approximately two to three orders of magnitude higher than pIRES2-EGFP co-transfected HEK293T cells (Table 2), which demonstrated that the recombinant phPPARγ-IRES2-EGFP plasmids were highly efficient in expression of hPPARγ mRNA levels in transfected HEK293T cells. Based on the fold change, the expression of hPPARγ seemed to fluctuate with different concentrations of rosiglitazone. Therefore, we performed a Pearson correlation test to determine that these fluctuations

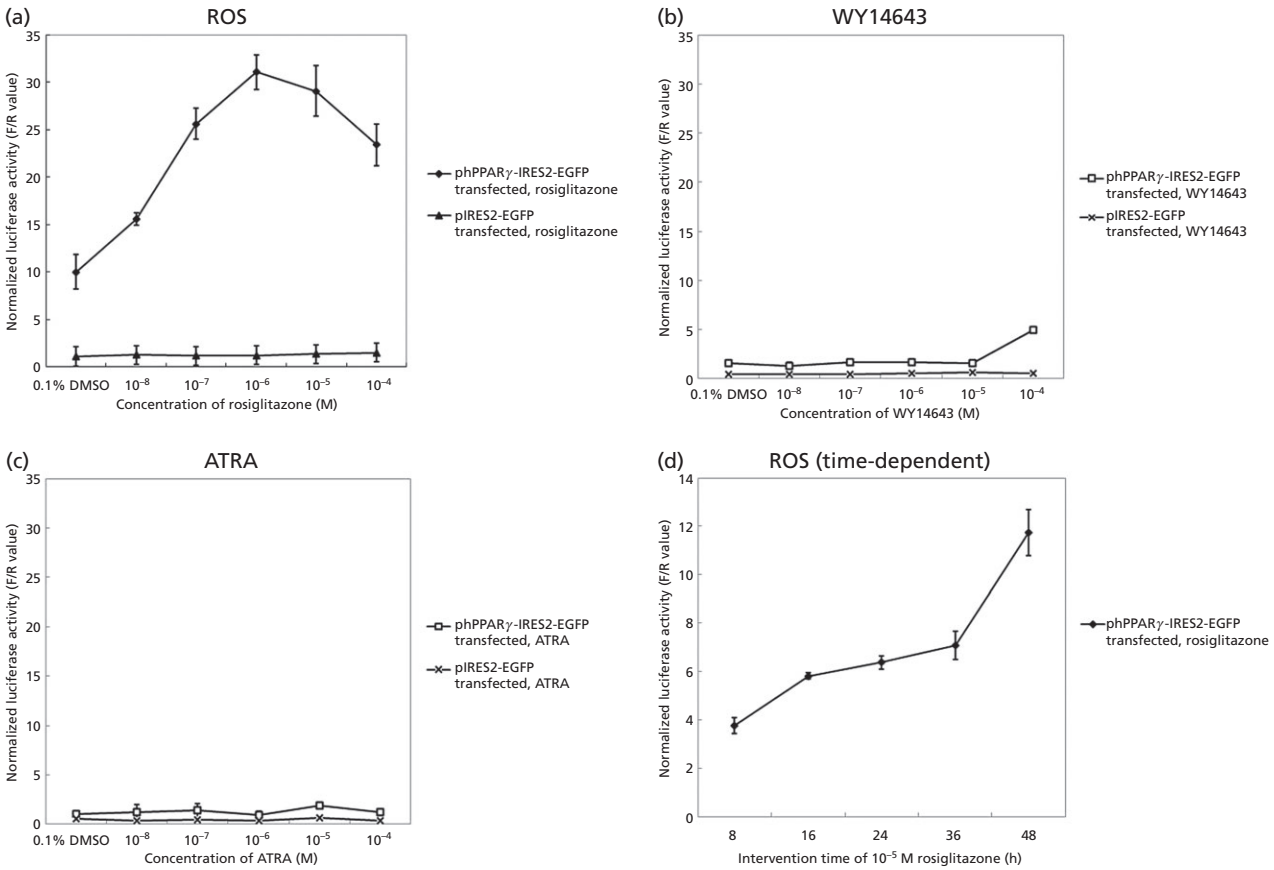

Figure 2 Luciferase expression activity in co-transfected HEK293T cells under different drug interventions. HEK293T cells were co-transfected with phPPARγ-IRES2-EGFP, pPPRE×3-TK-LUC, and pRL-CMV as indicated in 'Plasmids co-transfection'. Fourteen hours after transfection, the transfected mix was replaced with complete media and exposed to increasing doses of the peroxisome proliferator-activated receptor gamma (PPARγ) agonist rosiglitazone (ROS) (a), the peroxisome proliferator-activated receptor alpha (PPARα) agonist WY14643 (b), or the retinoic acid receptor alpha (RXRα) agonist all-trans-retinoic acid (ATRA) (c). DMSO, dimethyl sulfoxide. Cells were then collected 24 h after treatment, and the ratio of firefly to renilla luciferase luminescence (F/*R*-values) in each sample referred to as normalized luciferase activity were measured by dual luciferase assay. (d) Time course of the effects of 10^{-5} M rosiglitazone on co-transfected HEK293T cells. Each point was performed in triplicate, and the figure is a representative of three independent experiments. Error bars = SE.

Table 2 Comparison of hPPARγ mRNA expression levels in phPPARγ-IRES2-EGFP and pIRES2-EGFP transfected HEK293T cells treated with different doses of rosiglitazone

Group	phPPARγ-IRES2-EGFP/GAPDH (Ct value)	pIRES2-EGFP/GAPDH fold change($2^{-\Delta Ct}$)[a] *P* value (Ct value)
0.1% DMSO	15.96 ± 2.55/13.12 ± 0.64	25.27 ± 1.08/13.92 ± 0.11 364.56
10^{-8} M Rosiglitazone	11.23 ± 2.32/13.13 ± 0.54	23.98 ± 0.49/13.73 ± 0.77 4529.07 0.46
10^{-7} M Rosiglitazone	13.82 ± 1.00/12.56 ± 1.53	24.02 ± 0.21/11.92 ± 2.76 1833.01 0.52
10^{-6} M Rosiglitazone	14.22 ± 0.40/13.72 ± 0.24	25.18 ± 0.71/14.17 ± 0.16 1458.23 0.48
10^{-5} M Rosiglitazone	14.93 ± 0.29/13.20 ± 0.40	25.41 ± 0.53/14.32 ± 0.21 657.11 0.23
10^{-4} M Rosiglitazone	14.33 ± 0.21/14.10 ± 0.84	25.49 ± 0.23/14.85 ± 0.57 1351.18 0.47

DMSO, dimethyl sulfoxide; EGFP, enhanced green fluorescent protein. [a]Fold changes of human peroxisome proliferator-activated receptor gamma (hPPARγ) mRNA levels comparing phPPARγ-IRES2-EGFP transfected rosiglitazone-treated HEK293T cells with pIRES2-EGFP transfected HEK293T cells. The Ct values are expressed as the mean values of three independent experiments.

were not statistically significant ($r = -0.287$, $P > 0.05$), which suggested the expression of hPPARγ was not influenced by different concentrations of rosiglitazone. In addition, these results validated the low expression of hPPARγ in HEK293T cells itself. Melting curve and agarose gel electrophoresis confirmed that the products of real-time quantitative PCR were the specific hPPARγ gene fragments (Figure 3a).

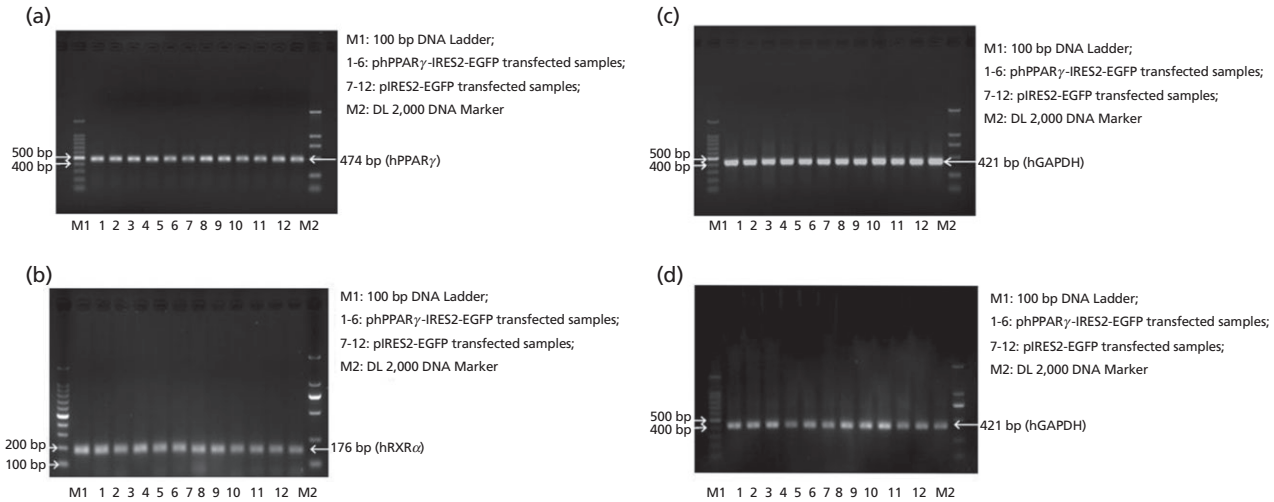

Figure 3 Electrophoresis of real-time PCR products of human peroxisome proliferator-activated receptor gamma (hPPARγ) and human retinoid X receptor alpha (hRXRα) in co-transfected HEK293T cells treated with different doses of PPARγ agonist rosiglitazone. RNA preparation, reverse transcription and real-time PCR were performed as indicated in Materials and Methods. For each sample, 200 ng total RNA was used for reverse transcription and polymerase chain reaction of hPPARγ or hRXRα. (a) Real-time PCR products of hPPARγ (474 bp). (b) Real-time PCR products of hRXRα (176 bp). (c), (d) Real-time PCR products of GAPDH (421 bp). M1: 100 bp DNA ladder; lane 1: 0.1% dimethyl sulfoxide (DMSO) in phPPARγ-IRES2-EGFP transfected 293T cells; lanes 2–6: rosiglitazone 10^{-4}–10^{-8} M in phPPARγ-IRES2-EGFP transfected HEK293T cells; lane 7: 0.1% DMSO in pIRES2-EGFP transfected HEK293T cells; lanes 8–12: rosiglitazone 10^{-4}–10^{-8} M in pIRES2-EGFP transfected 293T cells; M2: DL 2000 DNA maker. None of the samples showed genomic DNA amplification by melting point analysis (not shown).

Table 3 hPPARγ and hRXRα mRNA expression in phPPARγ-IRES2-EGFP and pIRES2-EGFP transfected HEK293T cells treated with different doses of rosiglitazone

Group	hPPARγ/GAPDH (Ct value)	hRXRα/ GAPDH (Ct value)	Fold contrast ($2^{-\Delta Ct(PPAR\gamma)}/2^{-\Delta Ct(RXR\alpha)}$)[a]	*P* value
phPPARγ-IRES2-EGFP	14.08 ± 1.58/13.30 ± 0.53	28.40 ± 0.46/13.15 ± 0.20	22 693.63	
pIRES2-EGFP	24.89 ± 0.70/13.82 ± 1.01	28.64 ± 0.26/13.11 ± 0.29	22.01	0.012

GAPDH, human glyceraldehyde-3-phosphate dehydrogenase. [a]Fold contrast comparing human peroxisome proliferator-activated receptor gamma (hPPARγ) to human retinoid X receptor alpha (hRXRα) mRNA levels in phPPARγ-IRES2-EGFP and pIRES2-EGFP transfected HEK293T cells. The Ct values are expressed as the mean ± standard error (SE) of series concentrations' rosiglitazone interventiona.

Quantitative real-time PCR results showed no appreciable difference between phPPARγ-IRES2-EGFP co-transfected HEK293T cells and pIRES2-EGFP co-transfected cells in the level of hRXRα mRNA. The hPPARγ mRNA expression level was four orders of magnitude higher than hRXRα mRNA expression level in phPPARγ-IRES2-EGFP transfected cells, and one order of magnitude higher than hRXRα mRNA expression level in pIRES2-EGFP transfected cells (Table 3), indicating that hRXRα expression was minimal in HEK293T cells. Melting curve and agarose gel electrophoresis confirmed the products of real-time quantitative PCR were the specific hRXRα gene fragments (Figure 3b).

Discussion

To date, the techniques of new drug high throughput screening consist of protein chip, polarized fluorescence, high content screening, model organisms, and cell models.[22–27] The cell models can simulate a variety of signalling pathways and imitate the in-vivo effects of drugs on human or animals, and so they are successfully used in drug screening studies and are also suitable for high-throughput screening.[28] Our previous study demonstrated that HEK293T cells expressed very lower hPPARγ in itself, and phPPARγ-IRES2-EGFP expression vector carrying human hPPARγ gene could be efficiently transfected and expressed in HEK293T cells.[18]

Due to these characteristics, we selected HEK293T cells as biological carriers and co-transfected these cells with a recombinant plasmid phPPARγ-IRES2-EGFP, and a firefly luciferase-containing report plasmid pPPRE×3-TK-LUC to imitate the hPPARγ signalling pathway. We determined whether this established hPPARγ signalling pathway model could be activated by the PPARγ agonist rosiglitazone using a dual luciferase reporter genes assay. Our results showed that the transcriptional activity of PPARγ in the cell model

could be activated by rosiglitazone in a dose- and time-dependent manner. To eliminate potential system error in the experimental procedure, we added plasmid pRL-CMV, which contains the renilla luciferase gene, in the co-transfected cell model as internal control. In addition, we optimized the experimental conditions for cell culture, transfection, the proportion of co-transfected plasmids, and detection time of reporter genes. Compared with other screening methods, the cellular drug screening model established here has an advantage of natural and specific response to PPARγ agonists. In addition to the application for new drug screening, this cell model can be used to compare the half maximal effective concentrations (EC50) of PPARγ ligands or agonists, and to evaluate the safety and activity of these PPARγ ligands as drug candidates. To our knowledge this unique function can not be achieved by other drug screening models.

Our data showed that phPPARγ-IRES2-EGFP was highly efficient in transfection and expression in the HEK293T cell model, which can fulfil the required expression levels of hPPARγ at the time of drug intervention. This study revealed that, under the intervention of different rosiglitazone concentrations, hPPARγ gene expression levels were stable. Thus, the expression of hPPARγ was not influenced by rosiglitazone. Double-luciferase assays showed that this cell model had a stable and specific reaction to PPARγ agonist rosiglitazone but failed to react to WY14643, a PPARα agonist. Since ligand-activated PPARγ forms a heterodimer complex with the RARα, we treated the model with ATRA, a RXRα specific agonist, to determine whether the model could be evoked through RXRα activation. The test results showed that the cell model could not be activated by ATRA. This interesting result may have been due to different recruitments of co-activator and co-repressor proteins for PPARγ/RXRα heterodimer when exposed to PPARγ or RXRα agonists and the exact molecular mechanisms need to be determined by further studies.

Recent studies have demonstrated that some active ingredients in traditional Chinese medicine are effective PPARγ agonists. Emodin, an active ingredient of rhubarb, could decrease body weight, blood lipids, hepatic triglyceride, and improve hepatic histological morphology in a rat model of nonalcoholic fatty liver.[29] The components and pharmacological effects of traditional Chinese medicine complex are complicated, identifying the best active ingredients has become a priority in contemporary studies of traditional Chinese medicine. Establishment of the cell model presented here may provide a simple and reliable method for drug screening of new hPPARγ agonists. Moreover, development of drug screening cell models specific to the desired signalling pathways not only have important advantages of screening new drugs with biological activity but can be used to study the mechanism of action of candidate drugs.

Conclusions

We successfully developed a new drug screening cell model targeting hPPARγ by imitation of the hPPARγ signalling pathway in co-transfected HEK293T cells. Compared with other drug screening methods, the current cell model can provide biological activity information and bridge the gap between the in-vitro assays and in-vivo studies. This effective and specific hPPARγ ligand agonist screening model opens up a new approach to screen candidates for hPPARγ agonists. Therefore, the cell model may be a useful tool in selection of new hPPARγ drugs that might be used in treatment of high blood cholesterol, obesity, hypertension, atherosclerosis, diabetes, cancer and other related diseases.

Declarations

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this manuscript and there are no conflicts of interest to declare.

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