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RNA interfering approach for clarifying the PPARγ pathway using lentiviral vector expressing short hairpin RNA

Kazufumi Katayama[a], Koichiro Wada[b,*], Hiroyuki Miyoshi[c], Kozo Ohashi[a], Masashi Tachibana[a], Rie Furuki[a], Hiroyuki Mizuguchi[d], Takao Hayakawa[d], Atsushi Nakajima[e], Takashi Kadowaki[f], Yasuo Tsutsumi[a], Shinsaku Nakagawa[a], Yoshinori Kamisaki[b], Tadanori Mayumi[a]

[a] *Department of Biopharmaceutics, Graduate School of Pharmaceutical Science, Osaka University, Osaka 565-0871, Japan*
[b] *Department of Pharmacology, Graduate School of Dentistry, Osaka University, 1-8 Yamadaoka, Suita, Osaka 565-0871, Japan*
[c] *Subteam for Manipulation of Cell Fate, BioResource Center, RIKEN, Tsukuba Institute, Ibaraki, Japan*
[d] *Division of Biological Chemistry and Biologicals, National Institute of Health Sciences, Tokyo 158-8501, Japan*
[e] *The Third Department of Internal Medicine, Yokohama City University School of Medicine, Yokohama 236-0004, Japan*
[f] *Department of Metabolic Diseases, Graduate School of Medicine, University of Tokyo, Tokyo, 113-0033, Japan*

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Abstract Peroxisome proliferator-activated receptor γ(PPARγ) plays a central role in adipocyte differentiation and insulin sensitivity. Although PPARγ also appears to regulate diverse cellular processes in other cell types such as lymphocytes, the detailed mechanisms remain unclear. In this study, we established a lentivirus-mediated short hairpin RNA expression system and identified a potent short hairpin RNA which suppresses PPARγ expression, resulting in marked inhibition of preadipocyte-to-adipocyte differentiation in 3T3-L1 cells. Our PPARγ-knockdown method will serve to clarify the PPARγ pathway in various cell types in vivo and in vitro, and will facilitate the development of therapeutic applications for a variety of diseases.

Key words: Peroxisome proliferator-activated receptor γ; RNA interference; Short hairpin RNA; Lentiviral vector; Adipocyte

1. Introduction

The peroxisome proliferator-activated receptor (PPAR) family was discovered as an orphan nuclear receptor, and three different subtypes were subsequently identified, namely PPARα, PPARδ/β and PPARγ. PPARγ is abundantly expressed in adipose tissue and plays a key role in adipocyte differentiation and insulin sensitivity [1]. Recently, our group and other researchers reported that PPARγ is also an attractive therapeutic target as it can play an important role in immune responses, especially in transcriptional regulation of inflammatory responses [2–5].

The biological role of PPARγ had been widely investigated by using PPARγ-deficient mice generated by targeted disruption of the PPARγ gene. Since homozygous PPARγ-deficient mice ($PPAR\gamma^{-/-}$) are embryonic lethal due to placental dysfunction [1], heterozygous mice ($PPAR\gamma^{+/-}$) have been used to investigate the role of PPARγ in vivo experiments. However, $PPAR\gamma^{+/-}$ mice seem to be of limited use in some experiments, because PPARγ also appears to regulate diverse cellular processes in cells that show lower levels of PPARγ expression in comparison to adipose tissue [6,7].

RNA interference (RNAi) is a powerful technique for selectively silencing the expression of genes. Recent work has provided a system for the stable expression of short interfering RNA (siRNA) in mammalian cells, which is generally based on the expression of short hairpin RNA (shRNA) under the control of the RNA polymerase III promoter [8–11]. The technique has allowed for the development of a new approach for achieving targeted gene silencing of disease-associated genes in animal models as well as in cultured cells.

Lentiviral vectors (LVs) are a promising tool for exogenous gene transfer among gene transfer vehicles, because LVs have the advantages of infecting non-dividing cells and being stably integrated into the host genome resulting in long-term expression of transgene [12–16]. Furthermore, recent reports have demonstrated that virus-mediated RNAi could provide long-term silencing in mammalian cells [9,17,18]. In the present study, we attempted to develop a technique for suppressing the expression of PPARγ in vivo and in vitro. We established a lentivirus-mediated shRNA expression system and identified a potent shRNA target sequence in the coding region of PPARγ mRNA. This approach has enabled us to clarify a novel role of PPARγ.

2. Materials and methods

2.1. Vector construction

Vectors were constructed using standard cloning procedures. H1-RNA promoter was amplified from human genomic DNA (Clontech, Palo Alto, CA, USA) using the following primers: 5′-CCATGGAATTCGAACGCTGACGTC-3′ and 5′-GCAAGCTTAGATCTGTGGTCTCATACAGAACTTATAAGATTCCC-3′. The amplified polymerase chain reaction (PCR) product was inserted into the *Eco*RI-*Bgl*II site of pHM5 [19], generating pHM5-H1. pHM5-H1 was designed to express shRNA upon the insertion of an appropriate sequence into the *Bgl*II/*Xba*I site (Fig. 1A). Oligonucleotides encoding

*Corresponding author. Fax: (81)-6-6879 2914.
E-mail address: kwada@dent.osaka-u.ac.jp (K. Wada).

Abbreviations: LV, lentiviral vector; shRNA, short hairpin RNA; MOI, multiplicity of infection; PPAR, peroxisome proliferator-activated receptor; GPDH, glycerol-3-phosphate dehydrogenase; BRL, rosiglitazone (BRL-49653)

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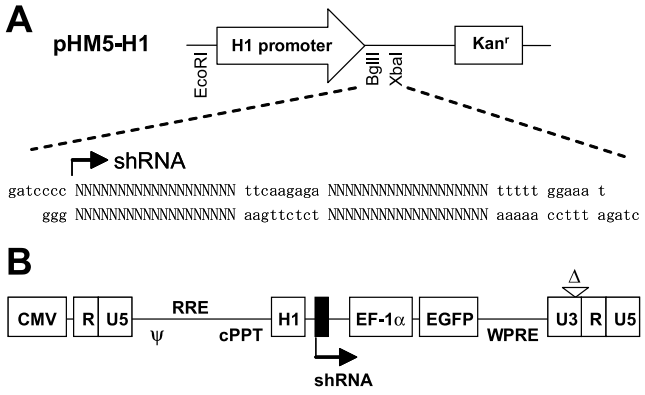

Fig. 1. Vector construction. A: pHM5-H1 was constructed as described in Section 2. Oligonucleotides encoding both strands of the targeting sequence, a spacer sequence which provided a loop structure and a transcriptional termination signal T5 were annealed and inserted into *Bgl*II/*Xba*I sites in pHM5-H1. B: Schematic representation of self-inactivating (SIN) LV plasmid (CS-H1-shRNA-EG). CMV: cytomegalovirus promoter, Ψ: packaging signal, RRE: rev responsive element, cPPT: central polypurine tract, H1: human H1 promoter, EF-1α: human elongation factor 1α subunit gene promoter, EGFP: enhanced green fluorescent protein, WPRE: woodchuck hepatitis virus posttranscriptional regulatory element. Δ: deleting 133 bp in the U3 region of the 3′ long terminal repeat.

both strands of the targeting sequence were annealed and inserted into *Bgl*II/*Xba*I sites of pHM5-H1 (Fig. 1A and Table 1). The sequence was verified on a DNA sequencer (ABI Prism 310, Applied Biosystems) and the cassette containing the H1 promoter plus the shRNA was transferred to a self-inactivating (SIN) LV construct, generating CS-H1-shRNA-EG (Fig. 1B).

2.2. Preparation of LV expressing shRNA (LV-shRNA)

LVs pseudotyped with vesicular stomatitis virus G glycoprotein (VSV-G) were prepared according to a previously described method [15,20,21]. Briefly, 293T cells were transfected with four plasmids: packaging construct (pMDLg/pRRE), VSV-G-expressing construct (pMD.G), Rev-expressing construct (pRSV-Rev), and SIN vector construct (CS-H1-shRNA-EG). Vector supernatant was concentrated by ultracentrifugation, and the pellet was resuspended in Hanks' balanced salt solution. Vector titers, which can be detected by enhanced green fluorescent protein (EGFP) expression under the control of a human elongation factor 1α subunit gene promoter, were determined by infection of HeLa P4 cells with serial dilutions of the vector stocks, followed by fluorescence-activated cell sorter (FACS) analysis for EGFP-positive cells.

2.3. Cell culture and infection of LV-shRNA

3T3-L1 preadipocytes were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The 3T3-L1 cells were infected with viral stocks at a multiplicity of infection (MOI) of 50 or 200, followed by FACS analysis for EGFP expression. Transduction efficiencies were 66.57% ± 1.44 at 50 MOI and 91.64% ± 1.07 at 200 MOI, expressed as S.E.M. The transduced 3T3-L1 cells were grown and then used in subsequent experiments.

2.4. Differentiation protocol

Induction of adipocyte differentiation was performed essentially as described [22]. Two days after confluence (day 0), the medium was replaced with differentiation medium containing rosiglitazone (BRL, 1 μM), insulin (INS, 150 nM), dexamethasone (DEX, 1 μM) and 3-isobutyl-1-methylxanthine (IBMX, 100 μM), which was changed every 3 days thereafter until analysis.

2.5. Measurement of adipocyte differentiation

Differentiation of 3T3-L1 preadipocytes to adipocytes was monitored by measurement of intracellular lipid accumulation using Oil red O staining and glycerol-3-phosphate dehydrogenase (GPDH) activity on day 9. Cultured cells were fixed for 2 h with 10% formalin in isotonic phosphate buffer and then washed with distilled water. The cells were then stained by complete immersion in a working solution (0.3%) of Oil red O for 4 h. Excess dye was removed by exhaustive washing with water. The GPDH activity was measured using a GPDH assay kit (Hokudo, Hokkaido, Japan).

2.6. RNA isolation and reverse transcription (RT) PCR analysis

Total RNA was extracted from the 3T3-L1 cells infected with each kind of LV-shRNA using Tri-Reagent (Sigma). First-strand cDNA was generated from 1 μg of RNA by using oligo(dT_{12-18}) primer (Invitrogen) and SuperScript III RNase H Reverse Transcriptase (Invitrogen) according to the manufacturer's protocol. The reverse transcription reaction mix was amplified with the following pair of oligonucleotides specific for murine PPARα, PPARδ, PPARγ2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH): PPARα, 5′-CGACAAGTGTGATCGGAGCTGCAAG-3′ and 5′-GTTGAAGTTCTTCAGGTAGGCTTC-3′; PPARδ, 5′-GGCCAACGGCAGTGGCTTCGTC-3′ and 5′-GGCTGCGGCCTTAGTACATGTCCT-3′; PPARγ2, 5′-GCTGTTATGGGTGAAACTCTG-3′ and 5′-ATAAGGTGGAGATGCAGGTTC-3′; GAPDH, 5′-GCTCACTGGCATGGCCTTC-3′ and 5′-ACCACCCTGTTGCTGTAGC-3′ [23]. The sample was amplified in the linear phase, optimized for each gene (PPARα: 38 cycles; PPARδ: 30 cycles; PPARγ2: 36 cycles; GAPDH: 23 cycles). All PCR products were electrophoresed on 2% agarose gel using 0.5×Tris-borate-EDTA buffer and visualized using ethidium bromide. The gel image was captured by a digital camera, and densitometric analysis was performed using NIH Image software.

2.7. Western blot analysis

Cultured cells were homogenized in Tris–HCl buffer containing a cocktail of protease inhibitors and insoluble materials were then removed by centrifugation at 4°C. The solubilized lysates were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis under reducing conditions at a concentration of 5 μg protein of sample per lane. Detection of PPARα, PPARδ, PPARγ and GAPDH was respectively performed with anti-PPARα polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-PPARδ polyclonal antibody (Santa Cruz Biotechnology), anti-PPARγ monoclonal antibody (Santa Cruz Biotechnology) and anti-GAPDH polyclonal antibody (Trevigen, Gaithersburg, MD, USA) according to a previously described method [4]. The band intensity was quantified using NIH Image software.

2.8. Statistical analysis

All results are expressed as mean ± S.E.M. Statistical comparisons were made with Student's *t*-test or Scheffé's method after analysis of variances. The results were considered significantly different at $P < 0.05$.

Table 1
LV vectors used in this study

LV vector	shRNA target gene	shRNA target sequence
LV-shRNA-P11	PPARγ1 and 2	CAGCTCTACAACAGGCCTC
LV-shRNA-P12	PPARγ1 and 2	ATGGCCATTGAGTGCCGAG
LV-shRNA-P13	PPARγ1 and 2	TAAATGTCAGTACTGTCGG
LV-shRNA-P14	PPARγ1 and 2	TTGGCGGAGATCTCCAGTG
LV-shRNA-P15	PPARγ1 and 2	GTCTGCTGATCTGCGAGCC
LV-shRNA-P16	PPARγ1 and 2	TCACCATTTGTCATCTACG
LV-shRNA-P17	PPARγ1 and 2	GTTTGAGTTTGCTGTGAAG
LV-shRNA-P18	PPARγ1 and 2	ATGAGCCTTCACCCCCTGC
LV-shRNA-P19	PPARγ1 and 2	GATCTGCGAGCCCTGGCAA
LV-shRNA-P21	PPARγ2	ACTCTGGGAGATTCTCCTG
LV-shRNA-P22	PPARγ2	CCTTCGCTGATGCACTGCC
LV-shRNA-Lu	Luciferase	ACGCTGAGTACTTCGAAAT
LV-shRNA-Scramble	No target gene	GCGCGCTTTGTAGGATTCG
LV-EG	–	–

LV-EG has no shRNA-expressing cassette. All vectors carry an EGFP-expressing cassette as a marker gene so that the cells transduced with LV-shRNAs can be identified by green fluorescence.

3. Results and discussion

To develop an effective PPARγ-knockdown method, we constructed an LV-based siRNA system in which shRNA encoding both strands of the targeting sequence is expressed under the control of human H1 promoter [24]. A human H1 promoter was cloned to generate pHM5-H1, and oligonucleotide encoding shRNA against PPARγ mRNA was inserted (Fig. 1A). Subsequently, the cassette containing the H1 promoter plus the shRNA was transferred to the SIN LV construct (Fig. 1B). Using a shRNA target sequence against firefly luciferase, we previously demonstrated that our LV-based siRNA system effectively suppressed the target gene in mammalian cells (data not shown).

PPARγ exists as two isoforms, termed PPARγ1 and PPARγ2,

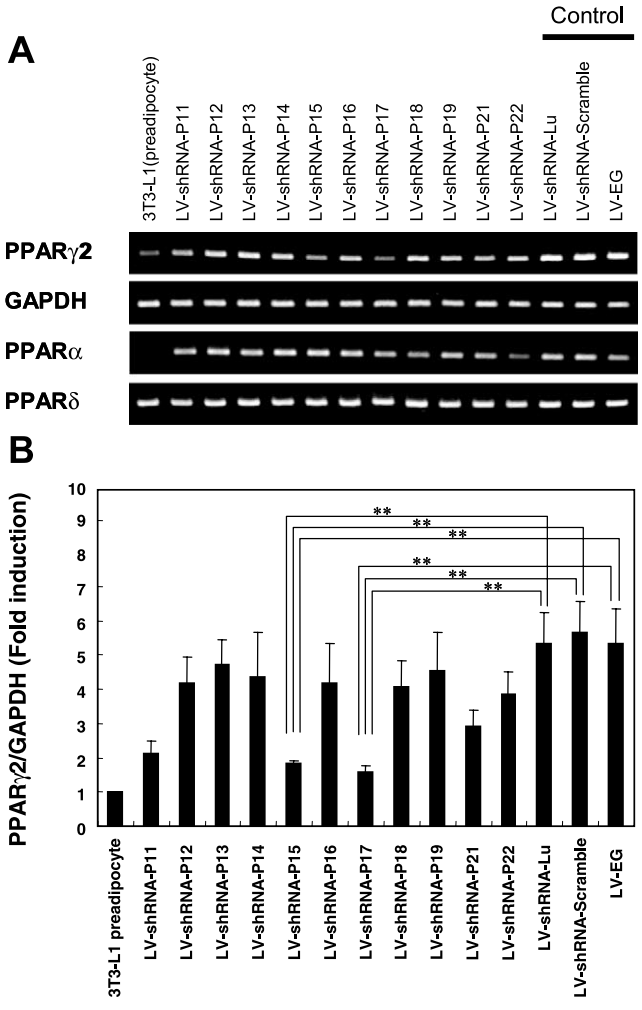

Fig. 2. Alteration of PPAR family mRNA levels in 3T3-L1 cells transduced with LV-shRNAs. A: 3T3-L1 preadipocytes were infected with each LV-shRNA (200 MOI) and then subjected to the differentiation protocol. Two days after the induction of adipocyte differentiation, mRNA levels of PPARγ2, PPARα, PPARδ, and GAPDH were determined by RT-PCR analysis. Results are representative gel images. B: Densitometric quantitation for PPARγ and GAPDH from three to four independent experiments. Each PPARγ value was normalized to the values for GAPDH and expressed as fold induction over the basal level detected in 3T3-L1 preadipocytes (bars, S.E.M.). $^{**}P < 0.01$ for LV-shRNA-P15 and -P17 compared with LV-shRNA-Lu, LV-shRNA-Scramble or LV-EG.

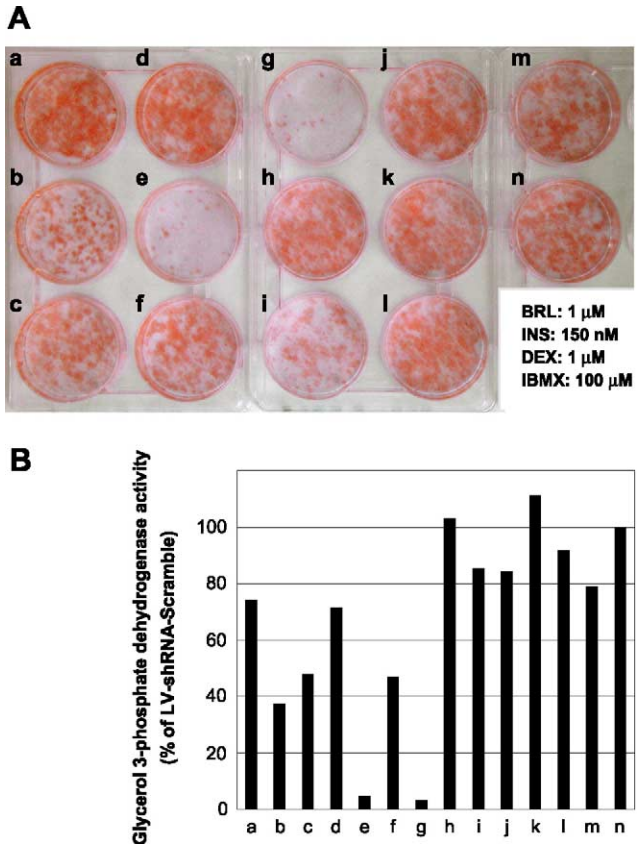

Fig. 3. Effect of LV-shRNAs on adipocyte differentiation. A: Differentiation of 3T3-L1 preadipocytes (infected with LV-shRNA; 200 MOI) to adipocytes was monitored by measurement of intracellular lipid accumulation using Oil red O staining on day 9. B: GPDH activity was measured on day 9. Data were expressed as percentage of the GPDH activity of 3T3-L1 cells which were infected with LV-shRNA-Scramble (200 MOI). a: LV-shRNA-P11; b: LV-shRNA-P12; c: LV-shRNA-P13; d: LV-shRNA-P14; e: LV-shRNA-P15; f: LV-shRNA-P16; g: LV-shRNA-P17; h: LV-shRNA-P18; i: LV-shRNA-P19; j: LV-shRNA-P21; k: LV-shRNA-P22; l: LV-EG; m: LV-shRNA-Lu; n: LV-shRNA-Scramble. Similar results were obtained in two independent experiments.

which are produced by a combination of different promoters and alternative splicing. PPARγ2 has an N-terminal extension of 30 amino acids and is very highly expressed in adipocytes [22,25]. We selected 11 target sequences in the coding region of PPARγ mRNA and constructed LV-shRNAs against PPARγ (Table 1). In the present study, LV-shRNA-Lu, LV-shRNA-Scramble and LV-EG were used as controls.

To find the most effective shRNA target sequence against PPARγ, we analyzed the silencing of PPARγ in 3T3-L1 cells during preadipocyte-to-adipocyte differentiation in which PPARγ is known to be a master regulator of adipogenesis [1,26,27]. The expression of PPARγ increases during the differentiation process and activation of PPARγ protein by its ligand leads to adipogenesis through the activation of the adipogenic gene cascade. The 3T3-L1 preadipocytes transduced with each of the LV-shRNAs, i.e. 3T3-L1 cells expressing shRNAs, as listed in Table 1, were exposed to differentiation medium (DM) 2 days after confluence (day 0). Initially, silencing of PPARγ expression was examined by RT-PCR after 2 days of culture in DM (Fig. 2). Although 3T3-L1 cells transduced with LV-shRNA-Lu, -Scramble and LV-EG showed

significant increases in the levels of PPARγ mRNA, 3T3-L1 cells transduced with LV-shRNA-P15 and -P17 retained low levels of PPARγ mRNA comparable to the level in preadipocytes maintained in normal culture medium. In contrast, the expression levels of GAPDH, PPARα and PPARδ were not altered by LV-shRNA-P15 or -P17. The other LV-shRNAs against PPARγ caused moderate decreases in the levels of PPARγ mRNA.

The differentiation of 3T3-L1 preadipocytes to adipocytes can be monitored by measurement of intracellular lipid accumulation and GPDH (an important enzyme in triglyceride synthesis) activity [28–30]. Intracellular lipid accumulation was dramatically reduced in the LV-shRNA-P15- and -P17-infected 3T3-L1 cells as shown by Oil red O staining (Fig. 3A, e: LV-shRNA-P15; g: LV-shRNA-P17). GPDH activity also demonstrated that LV-shRNA-P15 and LV-shRNA-P17 express a potent shRNA which suppresses PPARγ mRNA expression, resulting in marked inhibition of preadipocyte-to-adipocyte differentiation (Fig. 3B). We also confirmed that the expression of PPARγ-inducible genes, such as uncoupling protein-1 and adipocyte fatty acid binding protein, were inhibited in 3T3-L1 cells transduced with LV-shRNA-P15 and LV-shRNA-P17 in the presence of the PPARγ-specific ligand, BRL (unpublished data).

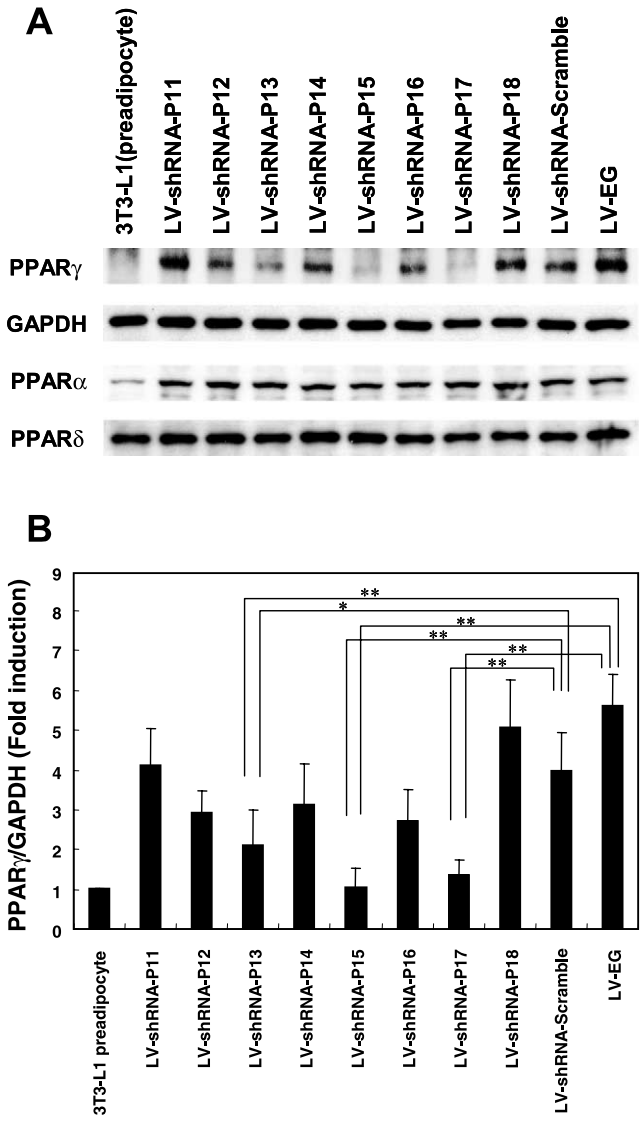

Fig. 4. Alteration of PPAR family protein levels in 3T3-L1 cells transduced with LV-shRNAs (200 MOI). A: Four days after the induction of adipocyte differentiation, the whole cell extract was analyzed by Western blotting with antibodies against PPARγ, PPARα, PPARδ and GAPDH. Results are representative of three individual experiments. B: Densitometric quantitation for PPARγ and GAPDH from three individual experiments. Each PPARγ value was normalized to the values for GAPDH and expressed as fold induction over the basal level detected in 3T3-L1 preadipocytes (bars, S.E.M.). $^{**}P < 0.01$ for LV-shRNA-P13, -P15 and -P17 compared with LV-shRNA-Scramble or LV-EG. $^{*}P < 0.05$ for LV-shRNA-P13 compared with LV-EG.

A recent study demonstrated that if the degree of complementarity to its target is reduced, siRNA can function as microRNAs which affect translational suppression without cleavage [31]. An important objective of this study was to determine whether the silencing effect of PPARγ caused by these LV-shRNAs was specific for PPARγ. In fact, several shRNA target sequences used in this study partially correspond to PPARα or PPARδ. Western blotting analysis demonstrated that PPARγ protein levels were significantly decreased in the LV-shRNA-P15- and LV-shRNA-P17-infected 3T3-L1 cells, while LV-shRNAs did not alter the amount of PPARα, PPARδ or GAPDH protein (Fig. 4). These results were consistent with the result from RT-PCR analysis (Fig. 2).

Furthermore, we examined 3T3-L1 cells exposed to either LV-shRNA-Scramble, -P15 or -P17 by fluorescent microscopy for EGFP expression to identify cells not infected with those vectors, i.e. the 3T3-L1 cells not expressing the shRNA encoded by LV-shRNA-P15 or -P17 (Fig. 5). In the case of LV-shRNA-Scramble, which expresses control shRNA, the differentiation of preadipocytes to adipocytes was not affected by infection with LV. In contrast, all of the cells infected with LV-shRNA-P15 or -P17 retained their fibroblast-like morphology. Taken together, these results indicate that our LV-shRNA-based PPARγ-knockdown method resulted in decreased PPARγ expression and specific inhibition of the PPARγ pathway, even in the case of adipocyte differentiation in which PPARγ expression is strongly induced by DM and PPARγ protein is effectively activated by the PPARγ-specific ligand used in this study, BRL.

Accessibility of the siRNA might depend on the secondary structure of the target mRNA. However, a clear correlation between either secondary structure or GC content and effectiveness of target sites has not yet been recognized. Although we designed 11 different shRNAs against PPARγ, we have not found any correlation between several factors that have been implicated in the accessibility of transcriptional/translational regulatory elements and effectiveness of target sites of shRNA until now.

In the present study, we developed a promising tool for suppressing the expression of PPARγ. Our PPARγ-knockdown method will serve to clarify the role of the PPARγ pathway in various cell types in vivo and in vitro, and will facilitate the development of therapeutic applications for a variety of diseases.

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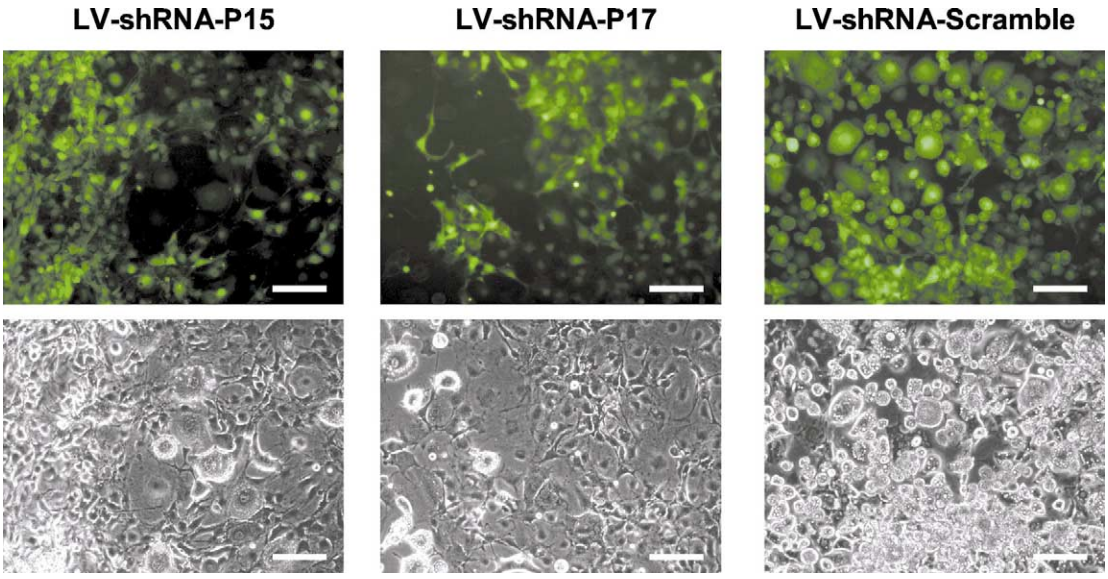

Fig. 5. Identification of the 3T3-L1 cells transduced with LV-shRNA. Brightfield and fluorescent microscopy images collected from the same field. The LV-shRNA-infected cells, which expressed EGFP, were detected as green fluorescence (upper panels) and morphologically identified mature adipocyte with a voluminous spherical shape and a large accumulation of intracytoplasmic lipid vesicles (lower panels). Bars represent 100 μm.

References

[1] Kubota, N. et al. (1999) Mol. Cell 4, 597–609.
[2] Su, C.G. et al. (1999) J. Clin. Invest. 104, 383–389.
[3] Desreumaux, P. et al. (2001) J. Exp. Med. 193, 827–838.
[4] Nakajima, A. et al. (2001) Gastroenterology 120, 460–469.
[5] Katayama, K. et al. (2003) Gastroenterology 124, 1315–1324.
[6] Schlezinger, J.J., Jensen, B.A., Mann, K.K., Ryu, H.Y. and Sherr, D.H. (2002) J. Immunol. 169, 6831–6841.
[7] Wang, Y.L., Frauwirth, K.A., Rangwala, S.M., Lazar, M.A. and Thompson, C.B. (2002) J. Biol. Chem. 277, 31781–31788.
[8] Brummelkamp, T.R., Bernards, R. and Agami, R. (2002) Science 296, 550–553.
[9] Abbas-Terki, T., Blanco-Bose, W., Deglon, N., Pralong, W. and Aebischer, P. (2002) Hum. Gene Ther. 13, 2197–2201.
[10] Hasuwa, H., Kaseda, K., Einarsdottir, T. and Okabe, M. (2002) FEBS Lett. 532, 227–230.
[11] Kunath, T., Gish, G., Lickert, H., Jones, N., Pawson, T. and Rossant, J. (2003) Nat. Biotechnol. 21, 559–561.
[12] Naldini, L., Blomer, U., Gallay, P., Ory, D., Mulligan, R., Gage, F.H., Verma, I.M. and Trono, D. (1996) Science 272, 263–267.
[13] Kafri, T., Blomer, U., Peterson, D.A., Gage, F.H. and Verma, I.M. (1997) Nat. Genet. 17, 314–317.
[14] Takahashi, M., Miyoshi, H., Verma, I.M. and Gage, F.H. (1999) J. Virol. 73, 7812–7816.
[15] Miyoshi, H., Smith, K.A., Mosier, D.E., Verma, I.M. and Torbett, B.E. (1999) Science 283, 682–686.
[16] Pfeifer, A., Kessler, T., Yang, M., Baranov, E., Kootstra, N., Cheresh, D.A., Hoffman, R.M. and Verma, I.M. (2001) Mol. Ther. 3, 319–322.
[17] Brummelkamp, T.R., Bernards, R. and Agami, R. (2002) Cancer Cell 2, 243–247.
[18] van de Wetering, M. et al. (2003) EMBO Rep. 4, 609–615.
[19] Mizuguchi, H. and Kay, M.A. (1999) Hum. Gene Ther. 10, 2013–2017.
[20] Tahara-Hanaoka, S., Sudo, K., Ema, H., Miyoshi, H. and Nakauchi, H. (2002) Exp. Hematol. 30, 11–17.
[21] Miyoshi, H., Blomer, U., Takahashi, M., Gage, F.H. and Verma, I.M. (1998) J. Virol. 72, 8150–8157.
[22] Tontonoz, P., Hu, E., Graves, R.A., Budavari, A.I. and Spiegelman, B.M. (1994) Genes Dev. 8, 1224–1234.
[23] Gimble, J.M., Robinson, C.E., Wu, X., Kelly, K.A., Rodriguez, B.R., Kliewer, S.A., Lehmann, J.M. and Morris, D.C. (1996) Mol. Pharmacol. 50, 1087–1094.
[24] Baer, M., Nilsen, T.W., Costigan, C. and Altman, S. (1990) Nucleic Acids Res. 18, 97–103.
[25] Chawla, A., Schwarz, E.J., Dimaculangan, D.D. and Lazar, M.A. (1994) Endocrinology 135, 798–800.
[26] Kliewer, S.A., Forman, B.M., Blumberg, B., Ong, E.S., Borgmeyer, U., Mangelsdorf, D.J., Umesono, K. and Evans, R.M. (1994) Proc. Natl. Acad. Sci. USA 91, 7355–7359.
[27] Tontonoz, P., Hu, E. and Spiegelman, B.M. (1994) Cell 79, 1147–1156.
[28] Ramirez-Zacarias, J.L., Castro-Munozledo, F. and Kuri-Harcuch, W. (1992) Histochemistry 97, 493–497.
[29] Green, H. and Kehinde, O. (1975) Cell 5, 19–27.
[30] Wise, L.S. and Green, H. (1979) J. Biol. Chem. 254, 273–275.
[31] Doench, J.G., Petersen, C.P. and Sharp, P.A. (2003) Genes Dev. 17, 438–442.