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Activation of p53 as a causal step for atherosclerosis induced by polycyclic aromatic hydrocarbons

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Abstract **This study was performed to prove our hypothesis that the metabolite(s) of polycyclic aromatic hydrocarbons (PAHs) caused the activation or phosphorylation of p53 via DNA damage to suppress the liver X receptor (LXR)-mediated signal transductions as a probably more direct mechanism. We found that LXR-mediated *trans*-activation was inhibited by 3-methylchoranthrene (MC) and doxorubicin (Dox) in HepG2 cells carrying wild-type p53, but not in Hep3B cells possessing mutant p53. The exogenous expression of wild-type p53 suppressed the LXR-mediated *trans*-activation in Hep3B cells. The expression of mRNA for ATP binding cassette A1 was suppressed by MC and Dox in HepG2 cells. The protein expression of retinoid X receptor (RXR), a partner of LXR to form a heterodimer, was suppressed by MC and Dox in HepG2 cells.**

Keywords: ABCA1; AHR; Atherosclerosis; DNA damage; Luciferase assay; MC; Quantitative RT-PCR

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), such as benzo[*a*]-pyrene (B[*a*]P) and 3-methylcholanthrene (MC), are ubiquitous contaminants in the environment. PAHs are detected in multiple sources, including cigarette smoke, exhaust emissions, industrial wastes and the pyrolysates of foods [1–3]. They cause a wide variety of toxicities, including carcinogenesis, atherogenesis and teratogenesis [4]. These toxic effects are known to be mediated by aryl hydrocarbon receptor (AHR), a ligand-dependent basic helix–loop–helix transcription factor [5–7]. A ligand-activated AHR translocates into the nucleus, forms a heterodimer complex with AHR nuclear translocator (ARNT), and finally interacts with xenobiotic responsive elements in the 5′-flanking regions of the AHR-target genes [8]. As one of the important mechanisms of PAH-induced toxicities, AHR up-regulates drug metabolizing enzymes such as cytochrome P450 (CYP), especially CYP1A1, which metabolizes PAHs to yield reactive intermediates causing DNA damage [9].

Tumor suppressor p53 is also known to be a transcription factor activated or phosphorylated by many types of stresses, including DNA damage [10,11]. The activated p53 subsequently *trans*-activates target-genes responsible for growth arrest or apoptosis [10,11]. Recently, it has been reported that p53 interacts with nuclear receptor, glucocorticoid receptor (GR), which is activated by glucocorticoids and maintains homeostasis in response to internal or external stresses [12]. The interaction results in the promotion of the proteosomal degradation of both proteins [12]. In addition, p53 is reported to inhibit the specific binding of nuclear receptor, androgen receptor (AR), which binds to androgens and is critical for the development, growth and maintenance of the male reproductive system, to DNA as a result of the inhibition of AR dimerization [12]. These studies provide evidence for a negative cross-talk between p53 and nuclear receptors.

PAHs, including B[*a*]P and MC, was reported to induce the atherosclerosis in several experimental animals [13–15]. Previously, we reported that MC inhibited liver X receptor (LXR)-mediated signal transductions, which are known to maintain cholesterol homeostasis, through AHR to cause atherosclerosis [16]. We also reported that the metabolism, probably the metabolic activation, of MC by CYP1A1 was a necessary step to repress the LXR-originated signal transductions by MC [17]. In the present study, we hypothesized that p53 activated by the metabolite(s) of PAHs suppressed LXR as well as GR or AR [12]. In this paper, we show evidence supporting our idea that p53 activated by PAHs acts as a negative regulator of LXR-mediated signal transductions to cause atherosclerosis via suppression of retinoid X receptor (RXR) expression, which is a partner of LXR to form a heterodimer.

2. Materials and methods

2.1. Cell culture

Human hepatoma-derived HepG2 and Hep3B cells were purchased from RIKEN (Tsukuba, Japan). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Nissui Pharmacy, Tokyo, Japan) supplemented with 10% fetal bovine serum (Bio Whittaker, Warkersville, MD), non-essential amino acids (ICN, Aurora, OH) and 1 mM sodium pyruvate (Gibco-BRL, Rockville, MD) in 5% CO_2 at 37 °C.

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Abbreviations: ABCA1, ATP binding cassette A1; AHR, aryl hydrocarbon receptor; AR, androgen receptor; ARNT, AHR nuclear translocator; B[*a*]P, benzo[*a*]pyrene; CYP, cytochrome P450; DMEM, Dulbecco's modified Eagle's medium; Dox, doxorubicin; GR, glucocorticoid receptor; LXR, liver X receptor; LXRE, LXR response element; MC, 3-methylcholanthrene; PAH, polycyclic aromatic hydrocarbon; RT-PCR, reverse transcriptase-polymerase chain reaction; RXR, retinoid X receptor; T1317, TO-901317; TK, thymidine kinase

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2.2. Plasmids

The p(LXRE)$_2$–thymidine kinase (TK)–Luc was constructed as described previously [16]. Full-length human p53 cDNA was obtained by PCR with a sense primer, p53-*Xho*I-S (5′-CGGGCTCGAGCCATGGAGGAGCCGCAGTC-3′), and an antisense primer, p53-*Xho*I-AS (5′-GTGGCTCGAGTCAGTCTGAGTCAGGCCCT-3′). The resultant fragment was digested with *Xho*I, and inserted into the *Xho*I site of the pcDNA 3.1 mammalian expression vector (pcDNA-p53) (Invitrogen, Carlsbad, CA).

2.3. Transient transfection and luciferase assay

One day before transfection, cells were plated at a density of 1×10^5 cells/well in a 12-well plate. HepG2 cells were transfected with 350 ng of p(LXRE)$_2$–TK–Luc, 100 ng of pcDNA-hLXRα and 50 ng of pRL-TK vector (as an internal control for transfection) by using Fugene6 (Roche Diagnostics, Indianapolis, IN). After the transfection, the medium was changed to fresh DMEM containing 1 μM TO-901317 (T1317), a LXR ligand (Sigma–Aldrich, St. Louis, MO), 1 μM MC, an AHR ligand (Sigma–Aldrich) and 1, 10 or 100 nM doxorubicin (Dox), a known p53 activator (Sigma–Aldrich). Hep3B cells were transfected with 350 ng of p(LXRE)$_2$–TK–Luc, 100 ng of pcDNA-hLXRα, 50 ng of pRL-TK vector and 0.1, 1 or 10 ng of pcDNA-p53. After the transfection, the medium was changed to fresh DMEM containing 1 μM T1317 and 0.1 or 1 μM MC. Cells were harvested after incubation for 36 h. Luciferase activity was measured using Dual-Luciferase Reporter Assay System (Promega, Madison, WI).

2.4. Real-time RT-PCR analysis

HepG2 cells were treated with 10 μM T1317, 10 μM MC and 100 nM Dox. After incubation for 24 h, total RNA from these cells was prepared using a GenElute Mammalian Total RNA Miniprep Kit (Sigma–Aldrich). Reverse transcription reaction was performed by using a First Strand cDNA Synthesis Kit for reverse transcriptase-polymerase chain reaction (RT-PCR) (AMV) (Roche Diagnostics). Quantitative real-time PCR was performed as described previously [16].

2.5. Western blot analysis

HepG2 cells were treated with 10 μM T1317 and 10 μM MC or 100 nM Dox. Nuclear extracts from these cells were prepared after incubation with MC for 0, 6, 12 or 24 h and with Dox for 24 h according to the method of Dignam et al. [18]. Protein concentration was determined using bovine serum albumin as a standard by BCA Protein Assay Reagent Kit (Pierce Biotechnology, Rockford, IL). Western blot analysis was performed using antibodies to LXRα (C-19), RXRα (D-20), p-p53 (Ser 15), p21 (C-19) (Santa Cruz Biotechnology, Santa Cruz, CA) and β-actin (AC-15) (Abcam, Cambridge, MA).

3. Results and discussion

To examine a possibility of whether the activation of p53 was a process critical for the suppression of LXR-mediated signal transductions, the effects of Dox, one of the representative p53 activators, on LXR-mediated transcriptional activity were investigated by a luciferase reporter assay using a reporter plasmid, p(LXRE)$_2$–TK–Luc (Fig. 1). When T1317, a LXR ligand, was added to a culture containing HepG2 cells transfected with the pcDNA-hLXRα,the luciferase activity seen with p(LXRE)$_2$–TK–Luc was elevated to a level of 12-fold higher than that of control (Fig. 1). The luciferase activity seen with p(LXRE)$_2$–TK–Luc in the presence of T1317 was decreased to a level of approximately 20–30% by co-treatment with MC (Fig. 1). Similarly, the luciferase activity was decreased by co-treatment with Dox in a dose-dependent manner (Fig. 1). These results support the idea that the activation of p53 is a process responsible for the suppression of LXR-mediated signal transductions by PAHs.

To further support the idea that p53 was involved in the suppression of LXR-mediated signal transductions by PAHs, the effects of MC and the exogenous expression of p53 on LXR-mediated transcriptional activity were investigated in Hep3B cells, a human hepatoma-derived cell line lacking wild-type p53 [19]. When T1317 was added to a culture containing Hep3B cells transfected with the pcDNA-hLXRα, the luciferase activity seen with the p(LXRE)$_2$–TK–Luc was increased to a level 15-fold higher than that of control (Fig. 2A). The co-treatment of Hep3B cells with MC did not affect the luciferase activity seen with the p(LXRE)$_2$–TK–Luc (Fig. 2A). When Hep3B cells were transfected with increasing amounts

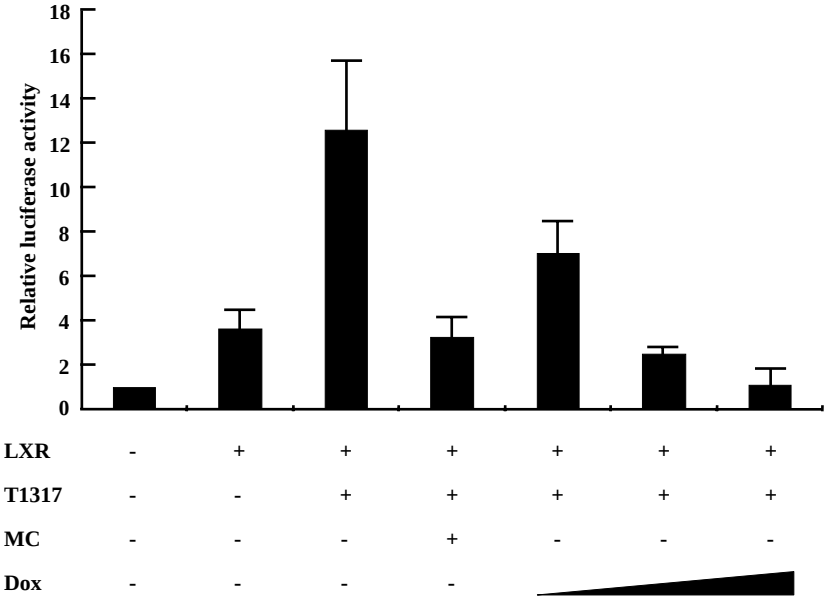

Fig. 1. Inhibition of LXR-mediated transcriptional activity by MC and Dox, which is the activator of p53. HepG2 cells were transfected with p(LXRE)$_2$–TK–Luc and pcDNA-hLXRα, and to the culture were added 1 μM T1317, 1 μM MC and 1, 10 or 100 nM Dox. Luciferase activity was measured after incubation for 36 h. Values in the figure represent the average ± S.D. from three independent experiments.

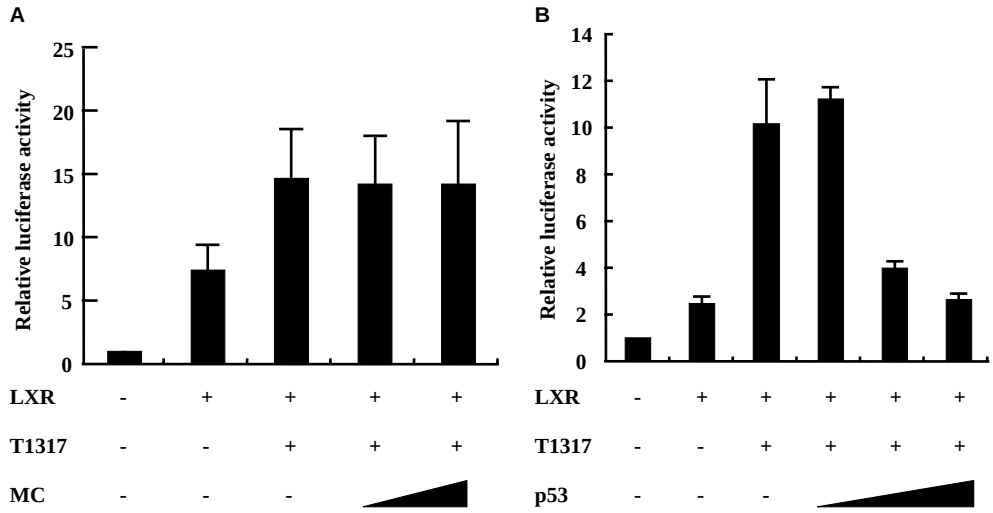

Fig. 2. Effects of MC and the exogenous expression of p53 on LXR-mediated transcriptional activity in Hep3B cells, hepatoma-derived cells carrying mutant p53. (A) A luciferase reporter plasmid, p(LXRE)$_2$–TK–Luc, was co-transfected into Hep3B cells with pcDNA-hLXRα. The Hep3B cells were treated with MC (0.1 or 1 μM). The luciferase activity was measured after incubation for 36 h. Values in the figure represent the average ± S.D. from three independent experiments. (B) A luciferase reporter plasmid, p(LXRE)$_2$–TK–Luc, was co-transfected into Hep3B cells with pcDNA-hLXRα and pcDNA-p53 (0.1, 1 or 10 ng). The luciferase activity was measured after incubation for 36 h. Values in the figure represent the average ± S.D. from three independent experiments.

of pcDNA-p53, the luciferase activity seen with p(LXRE)$_2$–TK–Luc was decreased depending on the amount of pcDNA-p53 (Fig. 2B). These results suggest that p53 plays a key role in the suppression of LXR-mediated signal transductions by PAHs.

To further support the results of reporter gene experiments, in which p53 was involved in the transcriptional down-regulation of the LXR-target genes, we examined the effects of Dox on the expression of mRNA for ATP binding cassette A1 (ABCA1), one of the LXR-target genes [20] (Fig. 3). The expression of ABCA1 mRNA was induced by treatment of HepG2 cells with T1317 and was suppressed by co-treatment with MC and Dox (Fig. 3).

It has been reported that p53 interacts with GR to promote the degradation of GR [12]. To examine the possibility of whether p53 promoted the degradation of LXR or its heterodimeric partner, RXR, the expression of LXR and RXR was investigated by western blot analysis (Fig. 4). Nuclear extracts were prepared from HepG2 cells after incubation for 0, 6, 12 or 24 h with MC, and 24 h with Dox. The increase in the amounts of phosphorylated p53 and p21 expression, which is known to be a typical p53-target gene, was seen after incubation for 6, 12 or 24 h with MC, indicating that p53 was activated by MC (Fig. 4). The expression of LXR was not decreased after incubation for 12 or 24 h with MC, while the notable decrease of RXR expression was found after incubation for 12 or 24 h with

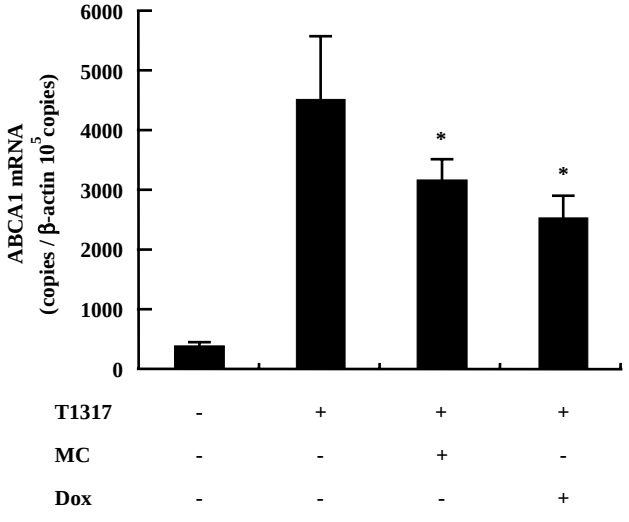

Fig. 3. Suppression of the expression of mRNA for ABCA1 by MC and Dox. The expression of mRNA for ABCA1 was quantified by a real-time RT-PCR. HepG2 cells were incubated with 10 μM T1317 and 10 μM MC or 100 nM Dox. After incubation for 24 h, total RNA was prepared from the cells and subjected to the real-time RT-PCR. Values in the figure represent the average ± S.D. from three independent experiments. *, Statistically different ($P < 0.05$) relative to the cells treated with T1317 alone.

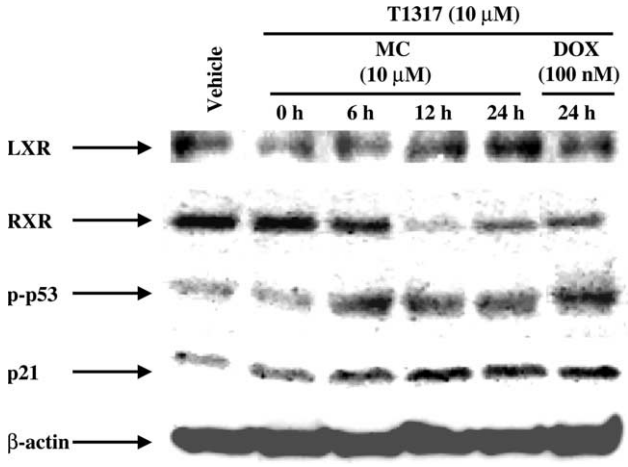

Fig. 4. Effects of MC and Dox on the protein expression of LXR and RXR. HepG2 cells were treated with 10 μM T1317 and 10 μM MC or 100 nM Dox. Nuclear extracts were prepared after incubation for 0, 6, 12 or 24 h with MC, or 24 h with Dox as indicated in the figure. Nuclear extracts (50 μg) prepared from the cells were subjected to SDS–PAGE and analyzed by western blot using antibodies to LXR, RXR, p-p53, p21 and β-actin.

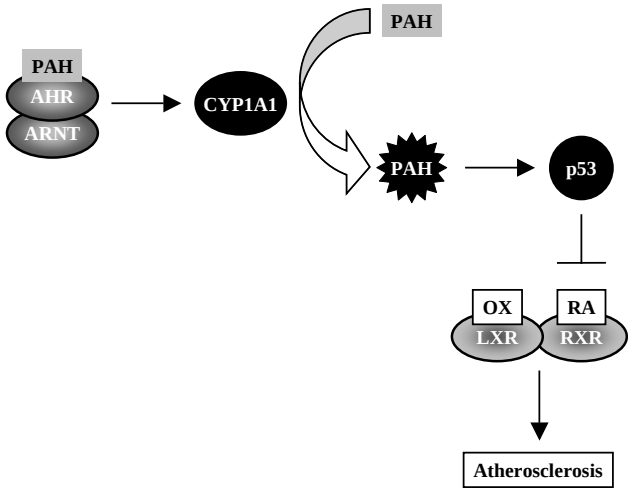

Fig. 5. Proposed mechanism(s) for the PAH-induced suppression of LXR-mediated signal transductions. PAH, polycyclic aromatic hydrocarbon; AHR, aryl hydrocarbon receptor; ARNT, AHR nuclear translocator; OX, oxysterols; RA, retinoic acid; LXR, liver X receptor; RXR, retinoid X receptor.

MC (Fig. 4). When HepG2 cells were treated with Dox for 24 h, the expression of RXR, but not LXR, was decreased (Fig. 4). These results suggest that the activated p53 suppresses the expression of RXR, which is a heterodimeric partner of LXR.

In the present study, we found that the activated p53 suppressed the expression of RXR to cause the suppression of LXR-mediated signal transductions. Yahagi et al. [21] reported that p53 and its target genes in adipocytes of *ob/ob* mice, which develop obesity, insulin resistance and glucose intolerance owing to an inherited deficiency of the appetite-suppressing hormone, were highly induced. They also found that the activation of p53 was responsible for the suppression of the lipogenic genes which were regulated by LXR. In addition, p53 is reported to negative-regulate nuclear receptors including GR and AR [12]. Together with these results, it may be possible to assume the mechanism of atherosclerosis induced by PAHs as follows (Fig. 5). First, PAHs bind to AHR and induce the expression of CYP1A1. Second, PAHs are metabolized by CYP1A1 to generate a reactive intermediate(s) and the resultant PAH-metabolites cause DNA damage to activate p53. Third, the activated p53 suppresses the protein expression of RXR, which is a heterodimeric partner of LXR. Finally, the expression of the LXR-target genes is suppressed to cause atherosclerosis.

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References

[1] Hattemer-Frey, H.A. and Travis, C.C. (1991) Benzo-a-pyrene: environmental partitioning and human exposure. Toxicol. Ind. Health 7, 141–157.
[2] Hecht, S.S. (1999) Tobacco smoke carcinogens and lung cancer. J. Natl. Cancer Inst. 91, 1194–1210.
[3] Knize, M.G., Salmon, C.P., Pais, P. and Felton, J.S. (1999) Food heating and the formation of heterocyclic aromatic amine and polycyclic aromatic hydrocarbon mutagens/carcinogens. Adv. Exp. Med. Biol. 495, 179–193.
[4] Miller, K.P. and Ramos, K.S. (2001) Impact of cellular metabolism on the biological effects of benzo[*a*]pyrene and related hydrocarbons. Drug Metab. Rev. 33, 1–35.
[5] Denison, M.S., Vella, L.M. and Okey, A.B. (1986) Structure and function of the Ah receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Species difference in molecular properties of the receptors from mouse and rat hepatic cytosols. J. Biol. Chem. 261, 3987–3995.
[6] Shimizu, Y., Nakatsuru, Y., Ichinose, M., Takahashi, Y., Kume, H., Mimura, J., Fujii-Kuriyama, Y. and Ishikawa, T. (2000) Benzo[*a*]pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. Proc. Natl. Acad. Sci. USA 97, 779–782.
[7] Silbergeld, E.K. and Gasiewicz, T.A. (1989) Dioxins and the Ah receptor. Am. J. Ind. Med. 16, 455–474.
[8] Hankinson, O. (1995) The aryl hydrocarbon receptor complex. Annu. Rev. Pharmacol. Toxicol. 35, 307–340.
[9] Ioannides, C. and Parke, D.V. (1993) Induction of cytochrome P4501 as an indicator of potential chemical carcinogenesis. Drug Metab. Rev. 25, 485–501.
[10] Vogelstein, B., Lane, D. and Levine, A.J. (2000) Surfing the p53 network. Nature 408, 307–310.
[11] Oren, M. (2003) Decision making by p53: life, death and cancer. Cell Death Differ. 10, 431–442.
[12] Sengupta, S. and Wasylyk, B. (2004) Physiological and pathological consequences of the interactions of the p53 tumor suppressor with the glucocorticoid, androgen, and estrogen receptors. Ann. NY Acad. Sci. 1024, 54–71.
[13] Albert, R.E., Vanderlaan, M., Burns, F.J. and Nishizumi, M. (1977) Effect of carcinogens on chicken atherosclerosis. Cancer Res. 37, 2232–2235.
[14] Majesky, M.W., Yang, H.Y., Benditt, E.P. and Juchau, M.R. (1983) Carcinogenesis and atherogenesis: differences in monooxygenase inducibility and bioactivation of benzo[*a*]pyrene in aortic and hepatic tissues of atherosclerosis-susceptible versus resistant pigeons. Carcinogenesis 4, 647–652.
[15] Paigen, B., Havens, M.B. and Morrow, A. (1985) Effect of 3-methylcholanthrene on the development of aortic lesions in mice. Cancer Res. 45, 3850–3855.
[16] Iwano, S., Nukaya, M., Saito, T., Asanuma, F. and Kamataki, T. (2005) A possible mechanism for atherosclerosis induced by polycyclic aromatic hydrocarbons. Biochem. Biophys. Res. Commun. 335, 220–226.
[17] Iwano, S., Asanuma, F., Nukaya, M., Saito, T. and Kamataki, T. (2005) CYP1A1-dependent mechanism for atherosclerosis induced by polycyclic aromatic hydrocarbons. Biochem. Biophys. Res. Commun. 337, 708–712.
[18] Dignam, J.D., Lebovitz, R.M. and Roeder, R.G. (1983) Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. 11, 1475–1489.
[19] Muller, M., Wilder, S., Bannasch, D., Israeli, D., Lehlbach, K., Li-Weber, M., Friedman, S.L., Galle, P.R., Stremmel, W., Oren, M. and Krammer, P.H. (1998) p53 activates the CD95 (APO-1/Fas) gene in response to DNA damage by anticancer drugs. J. Exp. Med. 188, 2033–2045.
[20] Repa, J.J., Turley, S.D., Lobaccaro, J.A., Medina, J., Li, L., Lustig, K., Shan, B., Heyman, R.A., Dietschy, J.M. and Mangelsdorf, D.J. (2000) Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. Science 289, 1524–1529.
[21] Yahagi, N., Shimano, H., Matsuzaka, T., Najima, Y., Sekiya, M., Nakagawa, Y., Ide, T., Tomita, S., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., Gotoda, T., Nagai, R., Kimura, S., Ishibashi, S., Osuga, J. and Yamada, N. (2003) p53 Activation in adipocytes of obese mice. J. Biol. Chem. 278, 25395–25400.