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Cell-type specific regulation of thrombospondin-1 expression and its promoter activity by regulatory agents

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Abbreviations: ATRA, all-*trans* retinoic acid; IFN-γ, interferon-γ; IL-6, interleukin-6; PMA, phorbol 12-myristate 13-acetate; PMSF, phenylmethyl sulfonyl fluoride; RARα, retinoic acid receptor α; TSP-1, thrombospondin-1

Abstract

Thrombospondin-1 (TSP-1), a multifunctional protein that is able to function as a negative regulator of solid tumor progression and angiogenesis, is normally present at a very low level but rapidly elevated in pathological tissues. To understand the cellular regulation of TSP-1 expression, the mode of it's expression in Hep3B, SK-HEP-1, and porcine aortic endothelial (PAE) cells was examined in the presence of all-*trans* retinoic acid (ATRA), interleukin-6 (IL-6), interferon-γ (IFN-γ), or phorbol 12-myristate 13-acetate (PMA). ATRA or IL-6 induced a dose-dependent increase of TSP-1 protein and mRNA levels in PAE cells, while they negatively regulated TSP-1 expression in the Hep3B and SK-HEP-1 cells. In contrast, PMA showed just the opposite effects on the TSP-1 expression in the (Hep3B and PAE) cells. IFN-γ had little effect on TSP-1 level in Hep3B and PAE cells. The TSP-1 expression in SK-HEP-1 cells by these agents showed a close resemblance to that of liver cells rather than that of the endothelial cell line. Possible TSP-1 promoter-mediated responses by ATRA, IL-6, IFN-γ, or PMA in Hep3B and PAE cells examined with luciferase activity of TSP-LUC reporter plasmid showed that levels of TSP-1 promoter activity were lower than that of the expressed TSP-1 protein and mRNA levels. Transfection of c-Jun and/or RARα expression vectors into Hep3B and PAE cells resulted in the enhanced TSP-1 promoter activity as well as the increments of of its protein and mRNA level. These results suggest that regulatory agents-induced TSP-1 expression may be attributed to mRNA stability and/or translational activation in concert with transcriptional activation and TSP-1 expression may be independently controlled via each signal pathway stimulated by PMA or ATRA.

Keywords: All-*trans* retinoic acid, IFN-γ, IL-6, PMA, TSP-1, Hep3B, PAE cell

Introduction

Thrombospondin-1 (TSP-1) is a homotrimeric glycoprotein synthesized and incorporated into extracellular matrix by numerous cells. TSP-1 is a multi-function protein known to regulate cell growth, motility, and apoptosis under physiological and pathophysiological states including wound healing, angiogenesis, and neoplasia (Lawler, 1986; Bornstein, 1992; Lahav, 1993; Roberts, 1996; Chen *et al*., 2000). Overexpression of TSP-1 suppresses tumor growth, but normal level is not sufficient to block tumor growth (Bluel *et al*., 1999; Streit *et al*., 1999). Furthermore, transfection of TSP-1 cDNA into a human breast carcinoma cell reduces primary tumor growth, metastatic potential, and angiogenesis (Weinstat-Saslow *et al*., 1994). TSP-1 level is inversely decreased to the increase of the proangiogenic factors, vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2) in human papilloma virus-16 positive cells (Bequet-Romero and Lopez-Ocejo, 2000). In the differentiation of neuroblastoma cells, TSP-1 production is rapidly induced by retinoic acid and acts as a mediator (Castle *et al*., 1992). Recently, a report has demonstrated that TSP-1 directly binds to gp120 of human immunodeficiency virus (HIV) in a concentration-dependent manner and blocks HIV infection (Crombie *et al*., 1998). Thus, the regulation of TSP-1 synthesis is of critical importance for modulating various biological processes and for new approaches in clinical therapy.

Generally, PMA increases TSP-1 synthesis in many different cells such as porcine thyroid cells, rat heart endothelial cells, and U937 cells, while it decreases in human peripheral blood monocytes (Verani *et al*., 1991c; Bellon *et al*., 1994; Yesner *et al*., 1996; Chua *et al*., 1997). In our previous reports, PMA up-regulates TSP-

1 expression in Hep3B and SK-HEP-1 cells, and down-regulates in PAE cells (Kim and Hong, 2000; Kim *et al.*, 2001). Although PMA exerts opposing effects on TSP-1 expression, c-Jun expression is consistently elevated in these cells (Kim and Hong, 2000; Kim *et al.*, 2001). We have observed that up-regulation of TSP-1 level induced by the retinoic acid in PAE cells is suppressed in human cell lines, Hep3B and SK-HEP-1 cells. Most of TSP-1 synthesized by cells is not circulating as free form, but rather binds to extracellular matrix and adjacent cells.

Retinoids consist of both natural and synthetic vitamin A derivatives. They exert their biological effects *via* two nuclear receptors, the retinoic acid receptors (RAR α, β, and γ), which bind with both all-*trans* retinoic acid (ATRA) and 9-*cis* RA, and the retinoid X receptors (RXR α, β, and γ), which only bind with 9-*cis* RA. The two classes of receptors are ligand-dependent transactivating factors that regulate gene expression by interacting with the promoter of target genes (Mangelsdorf *et al.*, 1992; Zhang *et al.*, 2000). They can also indirectly down-regulate the expression of certain genes by antagonizing the effect of AP-1 transcription factor formed by heterodimers of proteins of the c-jun and c-fos family (Schule *et al.*, 1991; Scita *et al.*, 1996).

SK-HEP-1 cell was derived from ascites fluid of a patient with hepatic adenocarcinoma and was initially characterized as a human hepatocarcinoma cell line such as Hep3B cells. However, it was recently recharacterized as an endothelial cell line because of its ability to express endothelial specific proteins such as endothelial leukocyte adhesion molecule-1 (ELAM-1), vimentin, and von Willebrand factor (Heffelfinger *et al.*, 1992). We investigated effects of IL-6 and IFN-γ that are known inducer of angiogenesis and inflammation (Fan *et al.*, 1992), along with ATRA and PMA on the expression of TSP-1 protein level. The results demonstrated that TSP-1 production in Hep3B, PAE, and SK-HEP-1 cells is regulated in a cell-type specific manner by ATRA, IL-6, IFN-γ, and PMA.

Materials and Methods

Cell stimulation and culture

PAE cells were provided by Korean National Institute of Health (KNIH) and maintained in M199 medium (Gibco Laboratories, Grand Island, NY) containing heat-inactivated 10% fetal bovine serum. Hep3B and SK-HEP-1 cells were purchased from the American Type Culture Collection (Rockville, MD) and cultured in RPMI 1640 medium containing heat-inactivated 10% fetal bovine serum. All cells were kept for 48 h at 37°C under 5% CO_2. To test the effects of regulatory agents, 2×10^5 cells/ml were plated on a 6- or 12-well culture plate with 2 ml or 1 ml media, respectively. Two days later, cells were washed twice with calcium/magnesium free-phosphate buffered saline (CMF-PBS, pH 7.2) and incubated at 37°C with serum free medium in the presence or absence of regulatory agent. The culture supernatants were harvested at the desired times and concentrated to determine the TSP-1 level. Two milliliters of culture supernatants were concentrated using Centricon-50 (Amicon, USA) at 3,000 *g* for 20 min in the presence of protease inhibitors, PMSF (1 μg/ml), leupeptin (1 μg/ml), and aprotinin (1 μg/ml). All experiments were carried out in duplicate or triplicate hereafter.

Determination of TSP-1 level by Western blot analysis

The concentrates were mixed with an equal volume of 2x sample buffer and electrophoresed through 7.5% SDS-polyacrylamide gel and transferred to nitrocellulose membrane. The membrane filter was blocked with 5% skim milk and incubated for 1.5 h with rabbit polyclonal anti-TSP-1 antibody (1:1000, Calbiochem, San Diego, CA). Antibody-treated membrane was washed four times for 5 min in each round with TBS buffer containing 0.1% Tween 20 (TTBS) and incubated with anti-rabbit peroxidase-conjugated antibody (1:4,000, Sigma Co., St. Louis, MO) for 1 h. After washing the membrane with TTBS, TSP-1 band was detected by enhanced chemiluminescence (ECL) detection system (Amersham, Buckinghamshire, U.K.).

Detection of TSP-1 mRNA by Northern blot analysis

Cells (2×10^5 cells/ml) were seeded onto 60 mm dishes. After two days, the cells were washed twice with CMF-PBS and incubated at 37°C with serum free media. Regulatory agents were added into the cultured cells and harvested at the indicated times. Total cellular RNA was isolated using RNA STAT-60 (TEL-TEST, INC., Friendwood, TX) and 10 μg of total RNA was electrophoresed through 1% agarose-formaldehyde gel electrophoresis. The electrophoresed RNAs were transferred to nylon membrane and hybridized with digoxigenin (DIG)-labeled probe. RNAs were visualized by immunochemical method using DIG chemiluminescence detection kit (Boeringer manheim, Germany). Probes for human TSP-1 was prepared as described (Kim and Hong, 2000). The cDNA probe of human β-actin was also prepared and hybridized as control.

Determination of transcriptional activities of TSP-1 promoter

First, 2974 bp of the promoter of the human TSP-1 gene was prepared from Hep3B genomic DNA by PCR with rTth DNA polymerase XL (PE Applied Biosystems, Foster City, CA) for providing reporter plasmid. The primers used were as followings, 5′-GAGGAAGGCTTTGTGT-TTGAGATA for the forward primer and 5′-ATCCTGTAG

CAGGAAGCACAAG for the reverse primer. PCR products were inserted into the Kpn I and Xho I sites of the promoterless pGL3 luciferase expression vector (Promega Co., Madison, WI). Amplification condition in PCR was previously described (Kim and Hong, 2000). For the transfection and reporter assay, Cells (3×10^5) were plated on a 6-well culture plate for 48 h and then transiently transfected with 2 μg plasmid DNA using FuGENE 6 (Boeringer Mannheim). The pTSP-LUC, pCR 3.1 encoding β-galactosidase, c-Jun expression vector pSG5, and RARα expression vector pSG5 were used in these transfections. Eight hours later, these cells were washed and treated with the respective agent. Twelve hours after treating agent, these cells were lysed with reporter lysis buffer (Promega Co., Madison, WI). Luciferase and β-galactosidase activities were measured. Luciferase activity was normalized to the β-galatosidase activity used as control of the transfection efficiency. Relative luciferase activity was expressed as mean ± SD in duplicate or triplicate. Statistical significance was evaluated using unpaired t-Test.

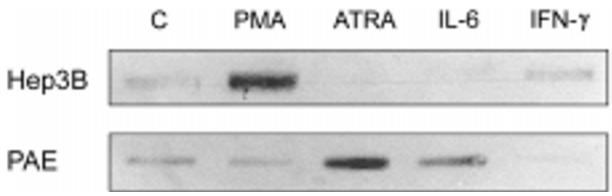

Figure 2. TSP-1 expression in Hep3B and PAE cells after treating various regulatory agents. Four regulatory agents were added at following concentrations, PMA, 50 nM; ATRA, 2 μM; IL-6, 100 U/ml; IFN-γ, 2 μg/ml. TSP-1 expression was diversely regulated by these agents in a cell-type specific manner. ATRA and IL-6 down-regulated in Hep3B cells, while they up-regulated in PAE cells. But PMA and IFN-γ-regulated TSP-1 expressions were inversely changed to those of ATRA and IL-6.

Results

Effects of regulatory agents on the TSP-1 level

To investigate the regulation of TSP-1 expression, Hep3B, SK-HEP-1, and PAE cells that express a sufficient level of TSP-1 protein were used and the culture media of each cells treated with regulatory agents; PMA, ATRA, IL-6, or IFN-γ were analyzed for TSP-1 by western blot analysis. ATRA or IL-6 abolished the expression of TSP-1 level in Hep3B and SK-HEP-1 cells in a dose-dependent manner, while markedly enhanced in PAE cells (Figure 1). In ATRA or IL-6-treated PAE cells, the level of TSP-1 began to increase at 0.5 μM ATRA or 10 U/ml IL-6 and reached the plateau level equivalent to 10-fold of control at 2 μM ATRA or 100 U/ml IL-6. Further increase of ATRA concentration (4 μM) caused decline of TSP-1 level. In SK-HEP-1 cell which expresses greater level of TSP-1 at the basal state responded at higher concentration of ATRA (1.5 μM) and IL-6 (100 U/ml). Cell specific expression of TSP-1 was demonstrated by PMA which induced up-regulation in Hep3B cells and down-regulation in PAE cells (Figure 2) and by IFN-γ which induced just a slight elevation in Hep3B cells and a decrease in PAE cells (Figure 2).

Regulation of TSP-1 transcription by regulatory agents

As shown in Figure 3, the levels of TSP-1 mRNA in both Hep3B and SK-HEP-1 cells were changed by ATRA (2 μM) and IL-6 (100 U/ml) in parallel with the level of TSP-1 protein. Transcriptional level started to decrease after 30 min and continued to decline steadily for 24 h in both cells. In PAE cells, ATRA and IL-6 induced up-regulation of TSP-1 transcription in steady state up to 6 h but decreased significantly at 24 h.

To determine whether the level of TSP-1 mRNA was resulted from the TSP-1 promoter activation, cells were transiently transfected with 2954 bp *Kpn* I/*Xho* I fragment of the TSP-1 gene containing –2,200 ~ +754 regions

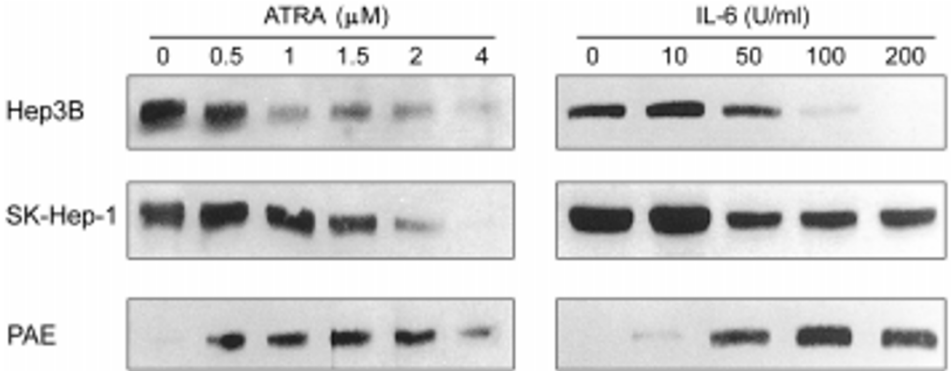

Figure 1. TSP-1 level secreted by ATRA or IL-6 stimulated Hep3B, SK-HEP-1, and PAE cells. Initially, 2×10^5 cells were cultured for 48 h in the media containing 10% serum and transferred to serum free media. Sequentially, cells in the serum free media were cultured for 48 h and the culture supernatants were analyzed by western blotting using rabbit polyclonal anti-TSP-1 antibody. The band of TSP-1 was visualized by ECL detection system.

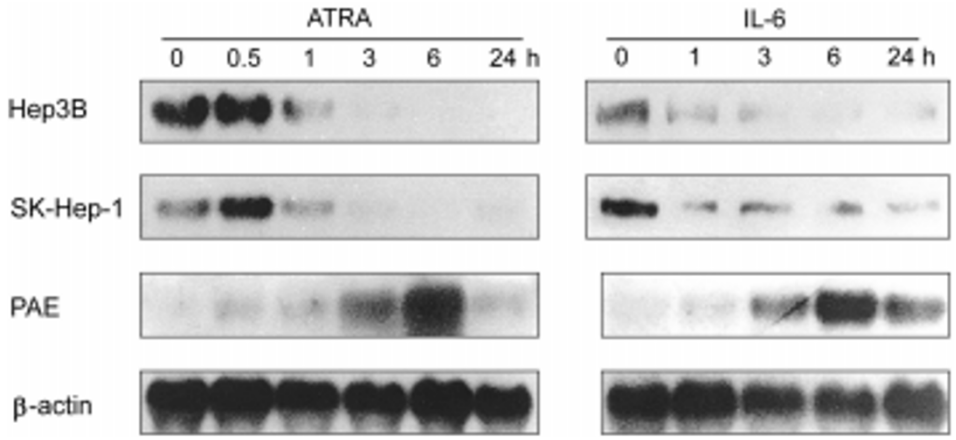

Figure 3. The mRNA level of TSP-1 synthesized by ATRA or IL-6-treated, Hep3B, SK-HEP-1, and PAE cells. ATRA and IL-6 were added into cells at the concentrations of 2 μM and 100 U/ml for the indicated time periods, respectively. The changed patterns of mRNA level were concordant with those of protein level in Figure 1. Ten μg of total RNA was used for Northern blotting. mRNAs for TSP-1 and β-actin were detected by hybridization with DIG-labeled cDNA probes and chemiluminescence reaction.

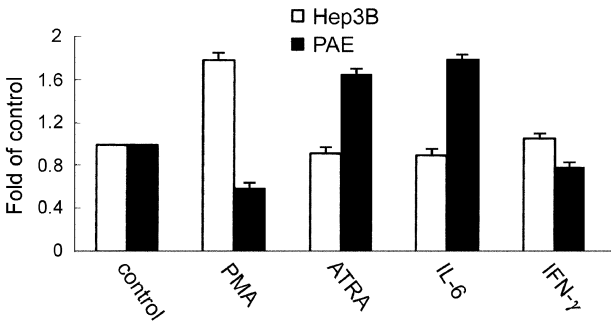

Figure 4. Transcriptional activities of TSP-1 promoter inserted into the luciferase reporter plasmid in various regulatory agents-stimulated Hep3B (open column) and PAE cells (closed column). TSP-1 promoter activities represent the relative activity to the luciferase activity without regulatory agents. All data were normalized for β-galactosidase and total protein, and expressed as the mean ± S.D. obtained from duplicate experiments. Bars on the column represent S.D.

fused into luciferase reporter plasmid. In Hep3B cells, transcriptional activity represented by TSP-1 promoter was enhanced to 1.8-fold by PMA, while it was less affected by IFN-γ (Figure 4). Moreover, ATRA or IL-6 also showed no effect on the TSP-1 promoter activity in contrast with the down-regulation of TSP-1 protein and mRNA level. In PAE cells, the exposure to 2 μM ATRA led to 1.7-fold increase in TSP-1 promoter-mediated luciferase activity (Figure 4). When PAE cells were treated with IL-6 (100 U/ml), the transcriptional activity of TSP-1 promoter was also increased to a similar level (about 1.8-fold) of the ATRA-induced increase. PMA (100 nM) in the PAE cells induced reduction of TSP-1 promoter activity to one-half level of the control, but IFN-γ showed no effect (Figure 4).

Regulation of TSP-1 expression in co-stimulation by PMA and ATRA

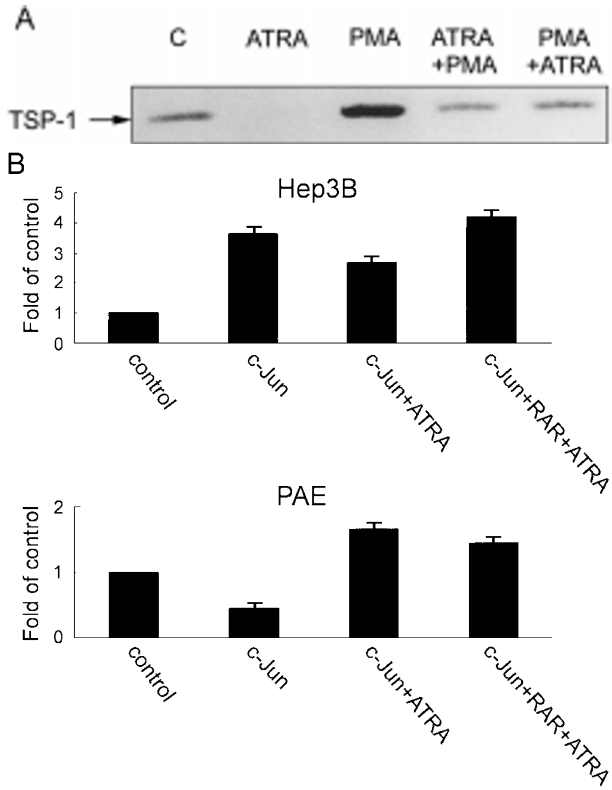

Figure 5. TSP-1 expression in the co-stimulated Hep3B cells with PMA and ATRA (A) and TSP-1 promoter activities induced by co-transfection with c-Jun and RARα expression vectors (B). To investigate the effects of ATRA on the PMA-induced increase of TSP-1 production in Hep3B cells (A), ATRA (2 μM) was treated for 30 min before or after adding PMA (50 nM). Superinduction of TSP-1 synthesis in PMA-treated Hep3B cells was slightly declined by ATRA. Furthermore, to examine whether the increase of TSP-1 expression by positive regulator was affected by negative regulator, c-jun and RARα expression vectors were co-transfected into Hep3B (upper Figure 5B) and PAE (bottom Figure 5B) cells, respectively. The luciferase activities were expressed as the relative activity to the control level obtained in the absence of regulatory agent. All data represent the means (bars S.D.) from two or three independent experiments.

As shown in Figure 5A, up-regulation of PMA-stimulated TSP-1 level in Hep3B cells was slightly suppressed by adding ATRA (2 μM). To demonstrate whether ATRA inhibited the PMA-induced positive regulation occurring through the transcriptional regulation of TSP-1 promoter, Hep3B (upper Figure 5B) and PAE (bottom Figure 5B) cells were transiently transfected with the TSP-LUC reporter plasmid in the combination with c-Jun and/or RARα expression vectors. As expected, c-Jun induced TSP-1 promoter activation in Hep3B cells. However, treatment of c-jun transfected cells with ATRA resulted in lower promoter activation. Co-transfection of Hep3B cells with c-jun and RARα expression vector together with ATRA treatment resulted in slight higher promoter activation. But in (PAE) cells, c-jun transfection induced suppression of TSP-1 promoter activation in comparison with control whereas RARα transfection plus ATRA treatment of (PAE) cells enhanced promoter activation. Such RARα-induced increase was slightly reduced by c-jun co-transfection in PAE cells.

Discussion

TSP-1 is ubiquitously synthesized and secreted but its level in body fluid is very low under normal physiological condition. Elevated TSP-1 level in the plasma or in the local sites was observed under pathological states and/or stimulation by regulatory agents (Lawler, 1986; Verani, 1991c; Ren and Savill, 1995; Bornstein, 1992; Chua *et al*., 2000). Understanding cellular regulation of TSP-1 expression may provide means to investigate modulation of numerous biological processes and may present insights into a possible approach to cancer therapy**.** In the present study, we have demonstrated that ATRA, IL-6, PMA, and IFN-γ diversely regulate TSP-1 protein and mRNA expression in a cell-type specific manner. The effects of ATRA on TSP-1 synthesis in various cells are controversial and differ significantly depending on the type of cells used. ATRA positively stimulates TSP-1 expression in HL-60, neuroblastoma, and human epidermal fibroblast cells, but negatively regulates in human squamous epithelial and arterial smooth muscle cells (Verani *et al*., 1991a & b; Castle *et al*., 1992; Touhami *et al*., 1997; Axel *et al*., 2001). Additionally, several evidences have demonstrated that TSP-1 synthesis is induced by ATRA during various biological processes including endothelial cell differentiation and consequent inhibition of angiogenesis (Jimenez *et al*., 2000; Nor *et al*., 2000). TSP-1 has been suggested to promote apoptosis of endothelial cells and inhibits angiogenesis by activating the caspase pathway (Jimenez *et al*., 2000; Nor *et al*., 2000).

IL-6 is known to be a potent stimulator for regulating acute phase response in liver cells. The effect of IL-6 on the TSP-1 expression is not known except that it exerts some effect in human umbilical vein endothelial cells (Loganadane *et al*., 1997). Our study demonstrated that ATRA and IL-6 markedly stimulate TSP-1 expression in PAE cells, but inhibit in Hep3B and SK-HEP-1 cells. TSP-1 synthesis in both transcriptional and translational level was inversely regulated by ATRA or IL-6 in Hep3B cells in comparison with those responses in PAE cells. Also, PMA exquisitely repressed the TSP-1 protein and mRNA level in PAE cells.

SK-HEP-1 cell, derived from ascitic fluid of a patient with hepatic adenocarcinoma has now been designated as an endothelial cell line by expression of characteristic endothelial-specific proteins, ELAM-1 and von Willebrand factor, and formation of capillary-like structure on Matrigel. In addition, Northern blot analysis of total cellular RNA from SK-HEP-1 cells shows no mRNA for the hepatic-specific proteins such as albumin, α- or γ-fibrinogen (Heffelfinger *et al*., 1992). However, our results suggest that SK-HEP-1 cells indeed possess partial characteristics of liver cells at least in TSP-1 expression.

IFN-γ is known to be a proinflammatory cytokine which exerts wide range of regulatory roles on cells. Current data indicate that it does not appear to modulate TSP-1 synthesis in any significant extent in PAE cells. IFN-γ treatment induced slight elevation of TSP-1 level in Hep3B cells but no effect in PAE cells. On the other hand, in human peripheral blood monocytes, INF-γ induced a significant enhancement of TSP-1 expression whereas it is repressed by PMA (Yesner *et al*., 1996). The newly synthesized TSP-1 proteins are not circulating as free form, but rather bind to the extracellular matrix and the neighboring cells (Wight *et al*., 1985). Here, we have demonstrated that TSP-1 expression was cell-type specifically regulated by various regulatory agents and likely the expressed TSP-1 could affect cells in various ways depending on the situations of local site.

In ATRA or IL-6-treated PAE cells, TSP-1 mRNA level initially observed gradually increased to reach maximal level at 6 h and maintained at a considerable level for 24 h. Such results that TSP-1 function as an immediate early responsive gene and last extended period may reflect its biological role. The immediate early gene family has been classified into three groups according to their kinetics in transcriptional responses to stimuli (Lau and Nathans, 1991). Group I genes show induction of mRNA synthesis within 30 min by stimulation of stimuli and reach peak level within 1 h. Group II gene mRNAs reach peak level about 2 h and rapidly return to control level thereafter. Group III gene display changes in transcription and mRNA accumulation that increase more slowly and maintain peak level for several hours. Thus, TSP-1 gene has intermediate characteristics between group I and group III.

In addition, the increase in the TSP-1 promoter acti-

vity was measured to be lower than that in the protein and mRNA synthesis of TSP-1. It means that TSP-1 expression by regulatory agents may be attributed to mRNA stability and/or translational activation in concert with transcriptional activation. In positive regulation of TSP-1 expression by PMA, its increased level was slightly reduced by ATRA or RARα transfection in Hep3B cells. In controlling TSP-1 expression by c-Jun and/or RARα transient transfection into both Hep3B and PAE cells, c-Jun and RARα exhibited opposing effects vise versa. These results suggest that TSP-1 expression is independently regulated through each signal pathway stimulated by PMA or ATRA. However, additional work will be required to confirm the detailed mechanism of TSP-1 expression at molecular level by a regulatory agent alone and by the combination of various regulatory agents.

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References

Axel DL, Frigge A, Dittmann J, Runge H, Spyridopoulos I, Riessen R, Viebahn R, Karsch KR. All-*trans* retinoic acid regulates proliferation, migration, differentiation, and extracellular matrix turnover of human arterial smooth muscle cells. Cardiovasc Res 2001;49:851-62

Bellon G, Chaqour B, Antonicelli F, Wegrowski J, Claisse D, Haya B, Borel JP. Differential expression of thrombospondin, collagen, and thyroglobulin by thyroid-stimulating hormone and tumor-promoting phorbol ester in cultured porcine thyroid cells. J Cell Physiol 1994;160:75-88

Bequet-Romero M, Lopez-Ocejo O. Angiogenesis modulators expression in culture cell lines positive for HPV-16 oncoproteins. Biochiem Biophys Res Commun 2000;277:55-61

Bluel K, Popp S, Fuseng NE, Stanbridge EJ, Boukamp P. Tumor suppression in human skin carcinoma cells by chromosome 15 transfer or thrombospondin-1 overexpression through halted tumor vasculature. Proc Natl Acad Sci USA 1999;96:2065-70

Bornstein P. Thrombospondins: structure and regulation of expression. FASEB J 1992;6:3290-99

Castle VP, Ou X, O'shea S, Dixit VM. Induction of thrombospondin 1 by retinoic acid is important during differentiation of neuroblastoma cells. J Clin Invest 1992;1857-63

Chen H, Herndon ME, Lawler J. The cell biology of thrombospondin-1. Matrix Biol 2000;19:597-614.

Chua CC, Hamdy RC, Chua BHL. Regulation of thrombospondin-1 production by angiotensin II in rat heart endothelial clls. Biochem Biophys Acta 1997;1357:209-14

Crombie R, Silverstein RL, Maclow C, Pearce S, Nachman RL, Lawrence J. Identification of CD 36-related thrombospondin 1-binding domain in HIV-1 envelope glycoprotein gp120: relationship to HIV-1 specific inhibitory factors in human saliva. J Exp Med 1998;187: 25-35

Fan TPD, Brem S, Cancer Biology Series. In The Search for New Anti-Cancer Drugs (Waring MJ, Ponder B, eds.). Vol. 3., 1992, pp.183-228, Boston, Mass

Heffelfinger SC, Hawkings HH, Barrish J, Taylor L, Darlington G. SK HEP-1: A human cell line of endothelial origin. In Vitro Cell Dev Biol 1992;28A:136-142

Jimenez B, Volpert OV, Crawferd SE, Febbraio M, Silverstein RL, Bouck N. Signals leading to apoptosis-dependent inhibition of neovascularization by thrombospondin-1. Natl Med 2000;6:418-48

Kim SA, Hong KJ. Responsive site on the thrombospondin-1 promoter to down-regulation by phorbol 12-myristate 13-acetate in procine aortic endothelial cells. Exp Mol Med 2000;32:135-40

Kim SA, Um SJ, Kang JH, Hong KJ. Expression of thrombospondin-1 in human hepatocarcinoma cell lines and its regulation by transcription factor Jun/AP-1. Mol Cell Biochem 2001;216:21-29

Lahav J. The functions of thrombospondin and its involvement in physiology and pathphysiology. Biochim Biophys Acta 1993;1182:1-4

Lau L, Nathans D. In The Hormonal Control of Gene Transcription (Cohen P. and Foulkes J.G., eds), 1991 Vol. 6, pp. 257-93 Elsevier, Amsterdam

Lawler J. The structural and functional properties of thrombospondin. Blood 1986;67:1197-209

Loganadane LD, Berge N, Legrand C, Fauvel-Lafeve F. Endothelial cell proliferation regulated by cytokines modulates thrombospondin-1 secretion into subendothelium. Cytokine 1997;9:740-46

Mangelsdorf DJ, Borgmeyer U, Heyman RA, Zhou JY, Ong ES, Ore AE, Kakizuka A, Evans RM. Chacterization of three RXR genes that mediate the action of 9-*cis* retinoic acid. Genes Dev 1992;6:329-44

Nor JE, Mitra RS, Sutorik MM, Mooney DJ, Castle VP, polverin PJ. Thrombospondin-1 induces endothelial cell apoptosis and inhibits angiogenesis by activating the caspase pathway. J Vasc Res 2000;37:209-18

Ren Y, Savill J. Proinflammatory cytokines potentiate thrombospondin-mediated phagocytosis of neutrophils undergoing apoptosis. J Immunol 1995;154:2366-74

Roberts DD. Regulation of tumor growth and metastasis by thrombospondin-1. FASEB J 1996;10:1183-91

Schule R, Rangarajan P, Yang N, Kliewer S, Ransone LJ, Bolado J, Verma IM, Evans RM. Retinoic acid is a negative regulator of AP-1-responsive genes. Proc Natl Acad Sci USA 1991;88:6092-96

Scita G, Darwiche N, Greenwald E, Rosenberg M, Politi K, De Luca LM. Retinoic acid down-regulation of fibronectin and retinoic acid receptor alpha proteins in NIH-3T3 Cells. J Biol Chem 1996;271:6502-08

Streit M, Velasco P, Brown LF, Skobe M, Richard L, Riccardi L, Lawler J, Detmar M. Overexpression of thrombospondin 1 decreases angiogenesis and inhibits the growth of human cutaneous squamous cell carcinomas. Am J Pathol 1999;155: 441-52

Touhami M, Fauvel-Lafeve F, Da Silva N, Chomienne C, Legrand C. Induction of thrombospondin-1 by all-*trans* retinoic acid modulates growth and differentiation of HL-60 myeloid leukemia cells. Leukemia 1997;11:2137-42

Verani J, Astrom A, Griffiths CE, Voorhees JJ. Induction of proliferation of growth-inhibited keratinocytes and fribroblasts in monolayer culture by sodium lauryl sulfate: comparison with all-*trans* retinoic acid. J Invest Dermatol 1991a;97:917-21

Verani J, Gibbs DF, Inman DR, Shah B, Fligiel SE, Voorhees JJ. Inhibition of epithelial cell adhesion by retinoic acid. Relationship to reduced extracellular matrix production and alteration. Am J Pathol 1991b;138:887-95

Verani J, Stoolman I, Wang T, Schuger L, Flipper C, Dame M, Johnson KJ, Todd RF 3rd, Ryan US, Ward PA. Thrombospondin production and thrombospondin-mediated adhesion in U937 cells. Exp Cell Res 1991c;195:177-82

Weight TN, Rougi GJ, Mumby SM, Bornstein P. Light microscopy immunolocation of thrombospondin in human tissues. J Hist Cytochem 1985;33:295-302

Weinstat-Saslow DL, Zabrensky VS, Van Hooutte K, Frazier WA, Roberts DD, Steeg PS. Transfection of thrombospondin1 complemetary DNA into a human breast carcinoma cell line reduces primary tumor growth, metastatic potential, and angiogenesis. Cancer Res 1994;54:6504-11

Yesner LM, Huh HY, Silverstein RL. Regulation of monocyte CD 36 and thrombospondin-1 expression by soluble mediators. Arterioscler Thromb Vasc Biol 1996;16:1019-25

Zhang D, Holmes WF, Wu S, Soprano DR, Soprano KJ. Retinoids and Ovarian Cancer. J Cell Physiol 2000;185:1-20