Involvement of thioredoxin-binding protein 2 in the antitumor activity of CD437

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The present authors previously reported that a synthetic retinoid, CD437, induces endoplasmic reticulum stress-mediated apoptosis in ovarian adenocarcinoma cells in spite of no response to natural retinoids. However, the precise mechanism of its proapoptotic action has not been fully determined. The present study herein demonstrates that apoptosis induction of ovarian adenocarcinoma SKOV3 cells by CD437 involves the upregulation of thioredoxin-binding protein 2 (TBP2) by a mechanism that is dependent on the intracellular calcium concentration. TBP2 is known to bind to and suppress thioredoxin (TRX) activity whereas TRX has an anti-apoptotic effect by inhibiting apoptosis signal-regulating kinase 1 (ASK1). The activation of ASK1 and its downstream molecule, c-Jun N-terminal kinase, was observed after induction of TBP2 by CD437. Interestingly, CD437 induced the association of TBP2 with TRX and, in turn, facilitated the dissociation of ASK1 from TRX. Moreover, blockade of TBP2 induction by small interfering RNA (siRNA) significantly attenuated the cytotoxic effect of CD437. These results suggest that TBP2 plays a critical role in the mechanism by which CD437 exerts proapoptotic action against SKOV3 cells. (*Cancer Sci* 2008; 99: 2485–2490)

Ovarian cancer remains the most malignant among gynecologic cancers, in part owing to the lack of specific marker enabling early detection, and its high recurrence rate.[1,2] In addition, early ovarian cancer is a silent and often asymptomatic disease.[2] Thus, it is often the case that patients complaining of symptoms have already developed ovarian cancer in stage III or IV at initial diagnosis.[2,3] The current standard treatments are surgical resection and chemotherapy. Except for some cancer types, ovarian cancer is generally regarded as chemosensitive, and the combination of paclitaxel and carboplatin is widely prescribed.[4] However, since the recurrence of chemoresistant ovarian cancer is often observed,[5] the prognosis of patients remains poor and the projected-5 years survival rate is still about 30%.[4] To improve the efficacy of chemotherapy against ovarian cancer, it is important to develop drugs with new mechanisms of action.

We previously reported that a synthetic retinoid, CD437, induced the upregulation of target genes of endoplasmic reticulum (ER) stress, including *CHOP* in ovarian adenocarcinoma SKOV3 cells, resulting in apoptosis in the cells.[6] Moreover, it was demonstrated that CD437-induced cytotoxic effect was partially prevented by small interfering RNA (siRNA)-mediated blockade of *CHOP* induction.[6] We further reported CD437-induced apoptosis in hepatocellular carcinoma cell lines via the mitochondrial pathway.[7] Although CD437 was first developed as a retinoic acid receptor (RAR) γ subtype-specific agonist,[8] several RAR-independent pathways such as c-Jun N-terminal kinase (JNK) pathway, may be involved in this action.[9,10] Interestingly, although another retinoid-related molecule ST1926 largely loses RAR transactivation activity,[11,12] ST1926 strongly induces apoptosis in cancer cell lines and it was proposed that ST1926 might share common mechanisms for apoptosis induction with CD437.[13]

Although clinical application of CD437 has been limited by its relatively unfavorable pharmacokinetic profiles and ST1926 has a more potent antitumor activity and more favorable pharmacokinetic characteristics than CD437,[11] CD437 efficiently inhibited the growth of ovarian cancer, melanoma and leukemia xenografts *in vivo* without significant toxicity[14–16] whereas no apoptosis induction by CD437 was observed in normal human epidermal keratinocytes and lung epithelial cells.[17,18] In addition, it was demonstrated that CD437 activates JNK more efficiently than ST1926 in the NB4 leukemia cell line.[13]

It has been reported that thioredoxin-binding protein 2 (TBP2) is involved not only in redox regulation by repressing thioredoxin (TRX) function,[19] but also in induction of apoptosis and growth arrest in cancer cells.[20] Moreover, it was found that TBP2 was epigenetically silenced in prostate and bladder cancer cell lines, whereas its expression was induced by histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA).[21] Thus, as such evidence piles up, TBP2 attracts attention as a tumor suppressor. On the other hand, TRX also plays a role in apoptosis regulation by binding to apoptosis signal-regulating kinase 1 (ASK1). Upon stimulation with oxidative stress, ASK1 is released from TRX and triggers apoptosis via JNK activation.[22,23]

To gain more insights into the mechanism of antitumor action of CD437, we focused on upstream signals for JNK activation, especially TBP2 and ASK1 in SKOV3 cells. We found that CD437 upregulated TBP2 expression, which bound to TRX and allowed ASK1 to be activated followed by JNK activation. Moreover, the apoptosis of SKOV3 cells by CD437 was significantly prevented by siRNA-mediated blockade of TBP2 induction. These results suggest that TBP2 plays a critical role in the mechanism of antitumor action of CD437.

Materials and Methods

WST assay. SKOV3 cells were cultured as previously reported.[6] WST assay was performed with TetraColor ONE (SEIKAGAKU, Tokyo, Japan). 5×10^3 cells were seeded in a 96-well plate. After overnight culture, cells were incubated with 0–1 μM CD437 (Sigma, St. Louis, MO, USA) for 72 h. Subsequently, the medium was replaced with 100 μL medium containing 5 μL TetraColor ONE. After 1 or 1.5 h of incubation at 37°C, the absorbances at 450 and 600 nm (as a reference) were measured. Cell viability at each concentration was expressed as the ratio to that of 0 μM CD437.

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RNA isolation and real-time reverse transcription-polymerase chain reaction (real-time RT-PCR). For mRNA analysis, cells were incubated in the absence or presence of CD437 for 0–12 h. For calcium chelation experiments, cells were treated with 1.0 μM of CD437 in the presence of a cell-permeable calcium chelator, *O*,*O*′-bis-(2-aminophenoxy)ethyleneglycol-*N*,*N*,*N*′,*N*′-tetraacetic acid tetraacetoxymethyl ester (BAPTA-AM; 20 μM, ABD Bioquest, Sunnyvale, CA, USA) for 8 h. Total RNA was recovered with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and subjected to reverse-transcription by using Superscript II (Invitrogen) and oligo(dT) primer. The level of TBP2 mRNA expression was determined by LightCycler System (Roche Diagnostics K.K., Tokyo, Japan) with primers (sense, 5′-AAGTAGTGGATCTGGTGGATGTCAATA-3′; antisense, 5′-CCGCCCATCAGGAATGAAC-3′). The expression level of the gene was normalized with that of β-actin (sense, 5′-GACGGCCAGGTCATCACTATTG-3′; antisense, 5′-CCACAGGATTCCATACCCAAGA-3′).

Measurement of intracellular calcium concentration. After washing cells twice with phosphate-buffered saline (PBS), SKOV3 cells on 10-cm dishes were incubated with 4 μM of Quest Fluo-8, AM cell permeable (Fluo-8; ABD Bioquest) in PBS for 20 min at 37°C. The cells were collected by scraping, and resuspended in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) at a concentration of $0.8–1 \times 10^7$ cells per mL. One hundred microlitres of the cell suspension were added onto a 96-well black plate and mixed with 100 μL of the culture medium containing CD437 and/or BAPTA-AM (final concentration; 1 and 20 μM, respectively). Following incubation at 37°C, fluorescence intensity was determined by a fluorescent microplate reader, and expressed as a relative to the values of dimethyl sulfoxide (DMSO)-treated controls at each time point.

Immunoprecipitation and Western blot analysis. For Western blot analysis, 3.5×10^5 SKOV3 cells were seeded in a 6-cm dish and allowed to attach overnight. CD437 was added to the medium and incubated for 0–12 h. Cells were lysed in RIPA buffer containing protease inhibitors (Roche Applied Science, Mannheim, Germany) and phosphatase inhibitors (Roche Applied Science).

For immunoprecipitation, 7.5×10^5 SKOV3 cells were seeded in three 10-cm dishes, and incubated in the presence of DMSO (control) or 1.0 μM CD437 for 12 h. Cells were collected and lysed in 100 μL NP40 buffer (1% NP-40, 50 mM Tris-HCl (pH 7.4), 150 mM NaCl) on ice for 30 min. The supernatants were recovered by centrifugation, and 5-μL aliquots of each sample were stored and used as input. Five micrograms anti-TRX antibody (MBL, Nagoya, Japan) was added into the residual aliquots (80 μL), and incubated overnight at 4°C with gentle rotation. Then, 50 μL of a 1:1 slurry of GammaBind G-sepharose beads (GE Healthcare UK, Buckinghamshire, England) was added and further incubated for 1 h at 4°C. Beads were washed three times with 1 mL of cold NP40 buffer, and suspended and boiled in 50 μL sodium dodecylsulfate (SDS) sample buffer. The supernatants were recovered by centrifugation, and subsequently subjected to Western blot analysis.

Primary antibodies used in Western blot analysis were as follows: TBP2 (JY-2) and human TRX (2E3) antibodies were purchased from MBL. Antibodies against ASK1, phospho-ASK1 (Thr845), JNK1/2/3, phospho-JNK1/2/3 (Thr183/Tyr185) and cleaved PARP (Asp214) were gained from Cell Signaling Technology (Beverly, MA, USA). Actin (I-19) antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

The intensity of the bands was quantified by using NIH Image software 1.63 (http://rsb.info.nih.gov/nih-image). Relative protein expression levels were normalized to the values of loading controls and expressed as ratios to non-treated samples.

Transfection with small interfering RNA and plasmid DNA. RNA interference (RNAi) duplexes for TBP2 (siTBP2; 5′-UUUCAGGGUCGUUAAAGACCACCUC-3′ and 5′-GAGGUGGUCUUUAACGACCCUGAAA-3′) and negative control (Negative Control Low GC Duplex) were purchased from Invitrogen. Plasmid DNAs harboring human TRX and TBP2 complementary DNA (cDNA) were previously reported.[19,24] enhanced green fluorescent protein (EGFP) expression vector was used as a negative control. SKOV3 cells on a 6-cm dish were transfected with RNAi duplexes and plasmid DNA using LipofectAMINE2000 (Invitrogen) and FuGENE6 (Roche Applied Science), respectively, according to the manufacturer's instructions. At 24 h post-transfection, cells were reseeded on 96-well plates, and then incubated in the presence of the indicated concentrations of CD437. Cell viability was examined by WST assay.

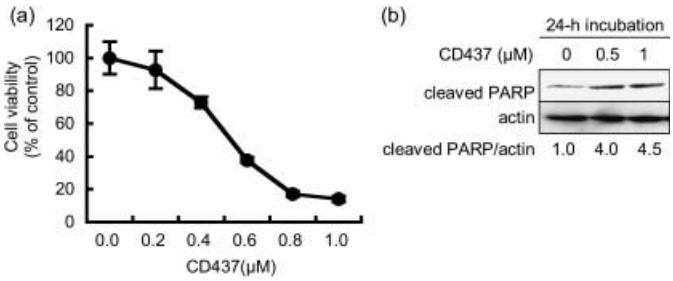

Fig. 1. CD437-induced apoptosis in SKOV3 cells. (a) Cell viability in the presence of CD437. WST assay was performed following 3-day treatment of SKOV3 cells with the indicated concentrations of CD437. Data are shown as means ± SD (n = 4). (b) CD437-induced cleaved PARP expression. Treatment with 0, 0.5 and 1.0 μM CD437 for 24 h was performed. Actin was used as an internal control. The ratios of band intensities of cleaved PARP to actin were shown.

Results

Induction of apoptosis in SKOV3 cells by CD437. As we previously reported, CD437 showed efficient reduction in the viability of SKOV3 cells with an IC_{50} of approximately 0.5 μM in this study (Fig. 1a). Moreover, the expression of cleaved PARP, which is indicative of apoptosis,[25] was increased in CD437-treated SKOV3 cells (Fig. 1b). Consistent with previous results, CD437 induced apoptosis of SKOV3 cells.

Involvement of TBP2 in CD437-induced growth inhibition of SKOV3 cells. Upon induction of apoptosis by CD437, the expressions of TBP2 mRNA and protein were upregulated in a time-dependent manner (Fig. 2a,b). As suggested by Garattini *et al.*,[15] increased intracellular calcium concentration is essential for triggering CD437- and ST1926-induced apoptosis. Measurement using a membrane-permeable fluorescent calcium indicator, Fluo-8, showed that intracellular calcium concentration ($[Ca^{2+}]_i$) of SKOV3 cells was transiently increased at 30 and 60 min following the treatment with CD437 (Fig. 2c). Moreover, membrane-permeable calcium chelator, BAPTA-AM, completely abrogated CD437-induced $[Ca^{2+}]_i$ elevation and TBP2 mRNA upregulation (Fig. 2c,d). These results suggest that free intracellular calcium is essential for CD437-induced TBP2 expression.

To investigate the involvement of increased TBP2 expression in the proapoptotic mechanism by CD437, SKOV3 cells were transfected with TBP2-specific siRNA, which abrogated CD437-induced TBP2 expression at both mRNA and protein levels (Fig. 3a,b). Interestingly, the knockdown augmented cell viability of SKOV3 cells treated with CD437 (Fig. 3c). Moreover, over-expression of TRX significantly increased the number of cells surviving in the presence of CD437, whereas that of TBP2 reduced the viability of SKOV3 cells (Fig. 3d). These results suggest that TBP2 is involved in the antitumor activity of CD437 by suppressing TRX function.

CD437-induced ASK1 activation. Since it has been reported that TRX functions not only as a redox regulator, but also a suppressor

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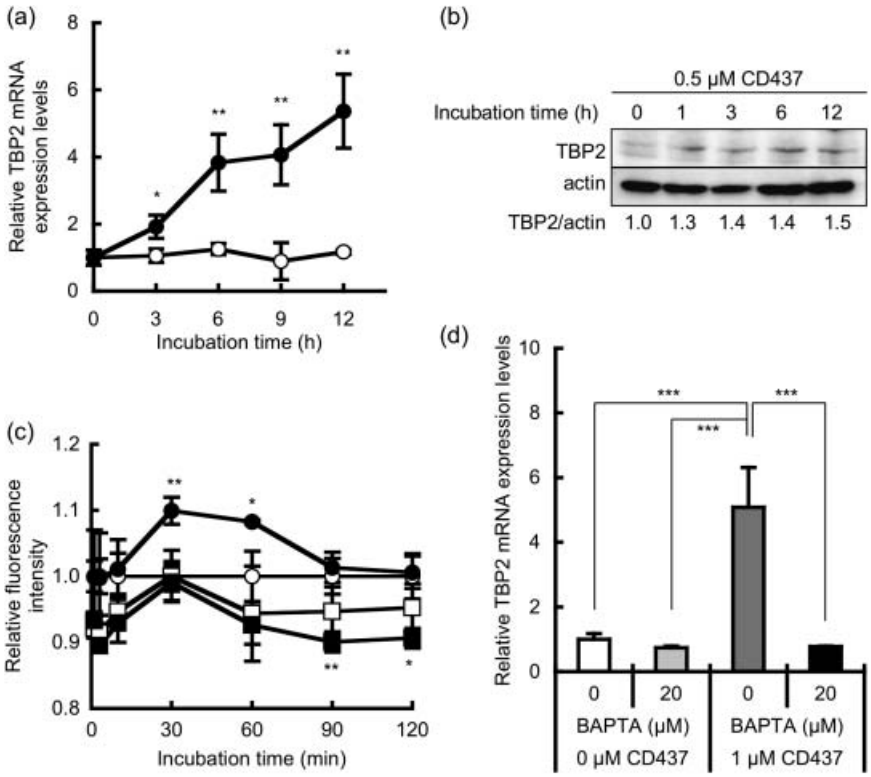

Fig. 2. CD437-induced thioredoxin-binding protein 2 (TBP2) upregulation. (a) CD437-induced TBP2 mRNA expression. Treatment was done with 0 (open circles) or 1.0 (closed circles) μM CD437 for the indicated periods of time. Expression level of TBP2 mRNA relative to that of β-actin was normalized by non-treatment control (0 h). Data are shown as means ± SD ($n = 3$). (*$P < 0.05$; **$P < 0.01$, *versus* non-treatment control). (b) CD437-induced TBP2 protein expression. Treatment with 0.5 μM CD437 for the indicated periods of time was done. Actin was used as an internal control. The ratios of band intensities of TBP2 to actin were shown. (c) Measurement of intracellular calcium concentration using Fluo-8. SKOV3 cells incorporated Fluo-8 were incubated with dimethyl sulfoxide (DMSO; open circles), 20 μM *O,O′*-bis-(2-aminophenoxy)ethyleneglycol-*N,N,N′,N′*-tetraacetic acid tetraacetoxymethyl ester (BAPTA-AM; open squares), 1 μM CD437 (black circles), or 20 μM BAPTA-AM and 1 μM CD437 (black squares) at 37°C for 1, 3, 10, 30, 60, 90, and 120-min. Relative fluorescence intensities of samples to those of DMSO-treated controls at each time point are shown as means ± SD ($n = 3$). (*$P < 0.05$; **$P < 0.01$, *versus* control). (d) Calcium chelator-mediated blockade of TBP2 mRNA induction. SKOV3 cells were incubated with DMSO (open column), 20 μM BAPTA-AM (light gray column), 1 μM CD437 (gray column), or 20 μM BAPTA-AM and 1 μM CD437 (black column) for 9 h. Expression level of TBP2 mRNA relative to that of β-actin was normalized by DMSO-treated control. Data are shown as means ± SD ($n = 4$). (***$P < 0.001$).

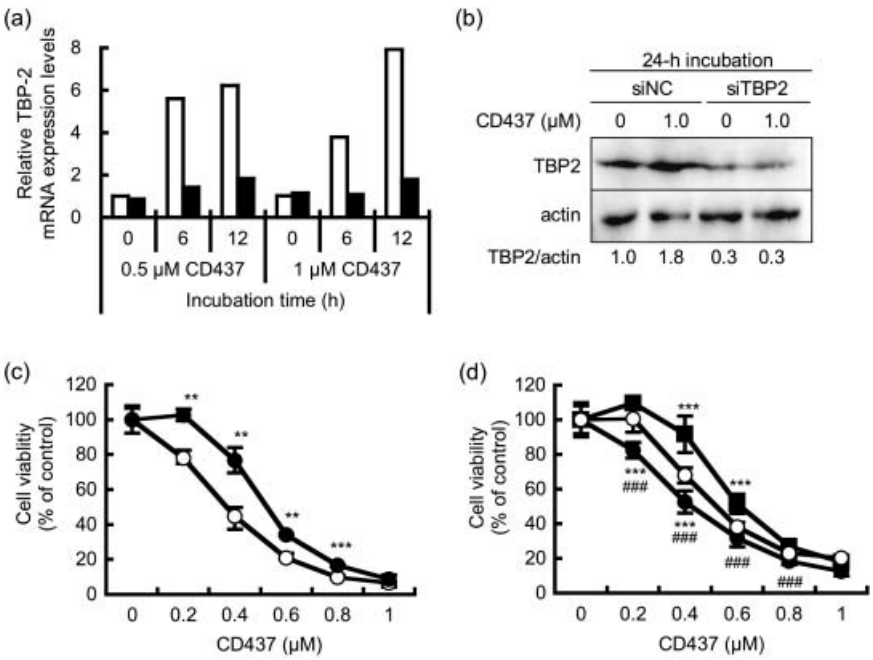

Fig. 3. The involvement of thioredoxin-binding protein 2 (TBP2) in antitumor effect of CD437. (a) Knockdown of TBP2 mRNA by small interfering RNA (siRNA). Following transfection with siRNA for negative control (open bars) and TBP2 mRNA (filled bars), cells were treated with 0.5 or 1.0 μM CD437 for 0, 6 and 12 h. (b) Knockdown of TBP2 protein by siRNA. Following transfection with siRNA for negative control (siNC) and TBP2 mRNA (siTBP2), cells were treated with 1.0 μM CD437 for 24 h. (c) Viability of TBP2-knocked-down cells in the presence of CD437. Following transfection with siRNA for negative control (open circles) or TBP2 mRNA (filled circles), SKOV3 cells were treated with the indicated concentrations of CD437 for 3 days. Cell viability was determined by WST assay. Data are shown as means ± SD ($n = 4$). (**$P < 0.01$; ***$P < 0.001$ *versus* negative control siRNA). (d) Viability of cells transfected with thioredoxin (TRX) or TBP2 expression vectors in the presence of CD437. Following transfection with plasmid DNA vectors expressing EGFP (negative control; open circles), TBP2 (filled circles) or TRX (filled squares), SKOV3 cells were treated with the indicated concentrations of CD437 for 3 days. Cell viability was determined by WST assay. Data are shown as means ± SD ($n = 4$). (***$P < 0.001$ *versus* negative control; ###$P < 0.001$ *versus* TRX expression vector).

of ASK1,[22] we examined whether ASK1 was activated by treatment with CD437. As expected, the expression of phosphorylated ASK1 was strikingly increased in time- and dose-dependent manners (Fig. 4a,b). This activation was completely abrogated by siRNA-mediated TBP2 knockdown (Fig. 4c). Moreover, JNK, downstream molecules of ASK1 signaling,[23] were also activated in time- and dose-dependent manners (Fig. 4d,e). Thus, CD437-induced apoptosis was mediated by TBP2–TRX–ASK1 pathway.

Finally, these results led us to further investigate the interaction of TRX with TBP2 and ASK1. By immunoprecipitation assay, it was indicated that although TRX bound to ASK1 in the absence of CD437, CD437 induced the association of TBP2 with TRX, and dissociated ASK1 from TRX (Fig. 5). Thus, it was suggested that activation of ASK1 was triggered by TBP2-mediated TRX suppression.

Discussion

We previously demonstrated that CD437 induced ER stress in ovarian adenocarcinoma and mitochondrial dysfunction in

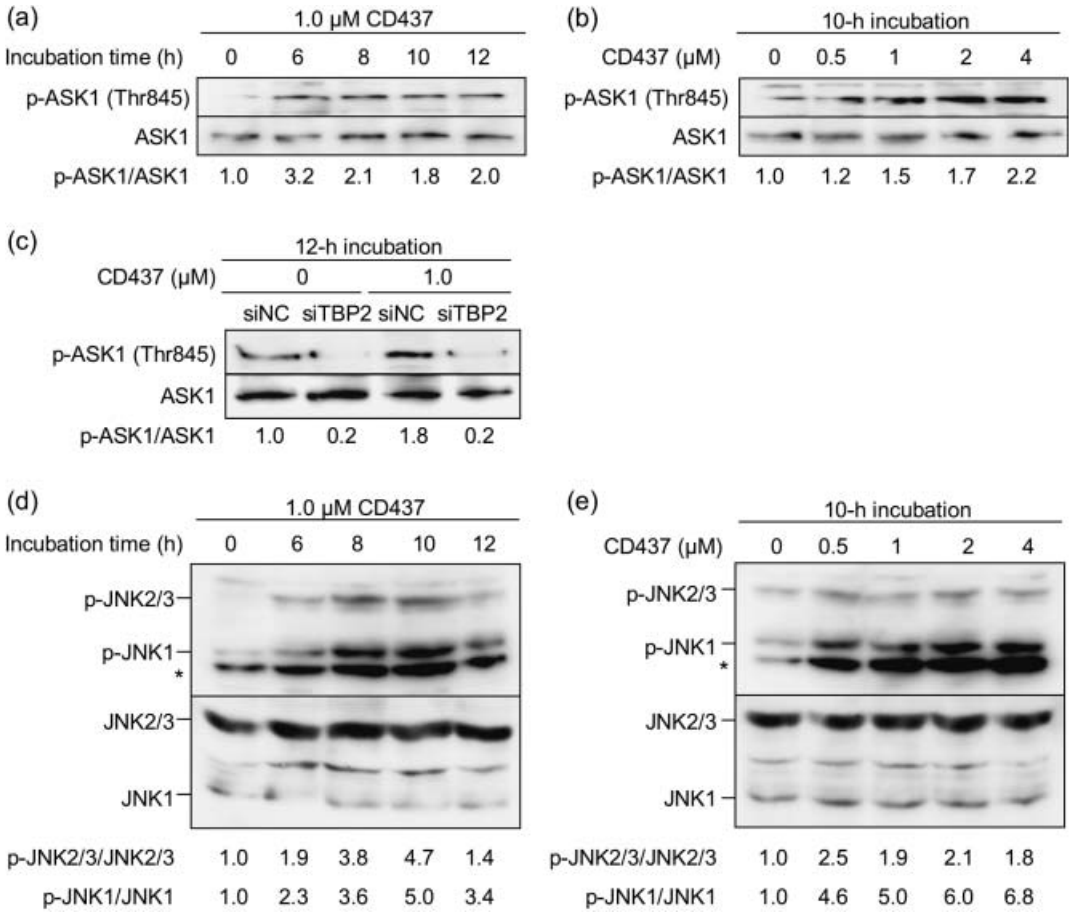

Fig. 4. CD437-induced activation of apoptosis signal-regulating kinase 1 (ASK1) signaling pathway. (a) Time-dependent ASK1 phosphorylation at Thr-845. The proteins from the cells treated with 1.0 μM of CD437 for the indicated periods of time were analyzed by Western blot. The ratios of band intensities of phosphorylated ASK1 (p-ASK1) to total ASK1 were shown. (b) CD437-induced dose-dependent ASK1 phosphorylation. The proteins from the cells treated with the indicated concentration of CD437 for 10 h were analyzed by Western blot. The ratios of band intensities of phosphorylated ASK1 (p-ASK1) to total ASK1 were shown. (c) Abrogation of CD437-induced ASK1 phosphorylation by thioredoxin-binding protein 2 (TBP2) small interfering RNA (siRNA). Following transfection with siRNA for negative control (siNC) or TBP2 mRNA (siTBP2), SKOV3 cells were treated with 0 or 1.0 μM of CD437 for 12 h. The ratios of band intensities of phosphorylated ASK1 (p-ASK1) to total ASK1 were shown. (d) Time-dependent c-Jun N-terminal kinase (JNK) phosphorylation. The proteins from the cells treated with 1.0 μM CD437 for the indicated periods of time were analyzed by Western blot. The ratios of band intensities of phosphorylated JNK (p-JNK) to total JNK were shown. Asterisk (*) indicates non-specific bands. (e) CD437-induced dose-dependent JNK phosphorylation. The proteins from the cells treated with the indicated concentration of CD437 for 10 h were analyzed by Western blot. The ratios of band intensities of phosphorylated JNK (p-JNK) to total JNK were shown. Asterisk (*) indicates non-specific bands.

hepatocellular carcinoma cell lines, leading to apoptosis in these cells.[6,7] To gain further insights into the molecular mechanism of CD437-induced apoptosis, we focused on the TBP2-mediated pathway as expression of TBP2 was strikingly upregulated by CD437. In the present study, TBP2 upregulated by CD437 binds to and suppresses TRX activity, allowing ASK1 to activate the JNK-mediated apoptosis signal pathway, as summarized in Figure 6.

CD437 was originally developed as a selective agonist of RARγ,[8] and was reported to induce the cytodifferentiation of F9 tetracarcinoma cells through this receptor.[12] However, it has been suggested that its mechanism of action, especially for apoptosis induction, does not involve the RAR-dependent pathway.[12,26] Indeed, in our previous study,[6] CD437 efficiently decreased the number of viable cells and induced apoptosis in SKOV3 cells while natural retinoids had no effects on cell viability in spite of RARγ expression on these cells. These data suggest that RARγ seems not to be prerequisite for the antitumor action of CD437. Moreover, TBP2 upregulation was not observed in the cells treated with all-*trans* retinoic acid (data not shown), suggesting that the mechanism of CD437-induced TBP2 expression also involves an RAR-independent pathway(s).

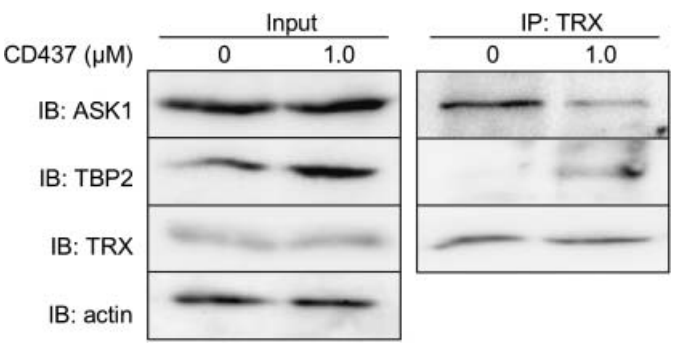

Fig. 5. Reciprocal interactions of thioredoxin (TRX) with apoptosis signal-regulating kinase 1 (ASK1) and thioredoxin-binding protein 2 (TBP2). The proteins from the cells treated with 0 or 1.0 μM CD437 for 12 h were immunoprecipitated with anti-TRX antibody. The whole cell extract was used as an input control.

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It has so far been reported that TBP2 expression was induced by various stresses including oxidative stress, ionizing radiation, heat shock,[27] high density cell culture,[27,28] and agents such as 1,25-dehydroxyvitamin D_3,[29,30] TGF-β1,[27,30] SAHA,[21] 5-fluorouracil,[31] PPARγ ligand[32] and ceramide.[33] TBP2 was first reported as vitamin D_3 upregulated protein 1 (VDUP1).[29] However, its function is likely unrelated to the physiological properties of vitamin D_3 since TBP2 was identified as a binding partner of TRX by yeast two-hybrid screen and suppresses TRX function in cellular redox regulation by selectively binding to the reduced form of TRX.[19] Moreover, ectopic expression of TBP2 induced cell-cycle arrest[24,30] and apoptosis[34] whereas TBP2 deficiency led to hepatocellular carcinoma in mice.[35] Recently, it was reported that TBP2 knockout mice developed features characteristic of human Reye-like syndrome, suggesting the involvement of TBP2 in lipid metabolism.[36] Thus, TBP2 is a multifunctional protein that is required for the maintenance of cellular homeostasis. Meanwhile, TRX also plays roles in not only redox regulation, but also suppression of ASK1-mediated apoptosis signaling, in which the reduced form of TRX binds to the N-terminal region of ASK1.[22] However, there have been a few reports linking between TRX–ASK1 and TRX–TBP2 interactions in apoptosis induction. Recently, it was reported that ceramide induces TBP2 expression, leading to apoptosis through ASK1 activation.[33] In the report, the dissociation of ASK1 from TRX was in parallel with ASK1 activation, suggesting that the suppression of TRX function by TBP2 is involved in the activation of ASK1-mediated apoptosis pathway. Although the interaction of TRX with TBP2 was not examined, these results strongly support the mechanism of CD437-induced growth inhibition proposed in this study (Fig. 6).

Recently, in addition to RARγ, it was reported that small heterodimer partner (SHP) plays a role as a nuclear receptor for CD437 and is involved in the apoptosis-inducing mechanism of CD437.[37] In the report, CD437 was shown to induce SHP-mediated c-Myc downregulation, resulting in apoptosis in leukemia and breast carcinoma cell lines.[37] It was reported that SHP is an orphan receptor consisting of a transcriptional corepressor complex, which was proposed to repress the ligand-dependent transcriptional activation of other nuclear receptors including RAR, RXR, thyroid hormone and estrogen hormone receptors.[38,39] Moreover, in contrast to these receptors, it was reported that the transcriptional activities of PPARα, PPARγ and NFκB were stimulated by SHP.[40,41] However, the relationship between transcriptional regulation of TBP2 and these nuclear receptors are still unknown. Although we demonstrated that the $[Ca^{2+}]_i$ elevation is essential for CD437-induced TBP2 expression, further study is required to clarify how TBP2 expression is regulated by CD437.

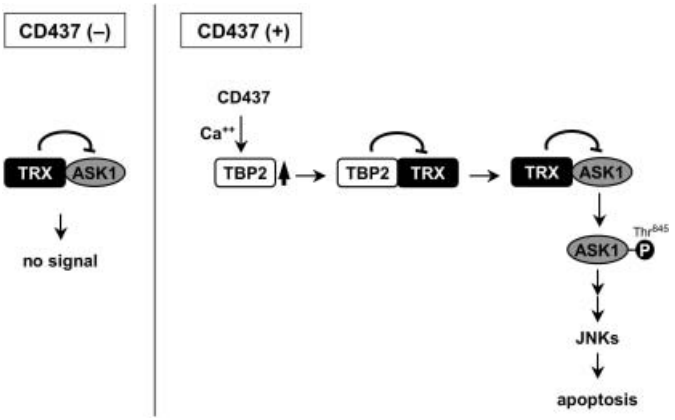

Fig. 6. Schematic representation of thioredoxin-binding protein 2 (TBP2)-mediated antitumor action of CD437. CD437 induces TBP2 expression and its interaction with thioredoxin (TRX), and disturbs TRX–apoptosis signal-regulating kinase 1 (ASK1) interaction, which is required for the suppression of ASK1-mediated apoptosis. This dissociation triggers ASK1 phosphorylation at Thr-845, which further facilitates c-Jun N-terminal kinase (JNK) activation, resulting in apoptosis in SKOV3 cells.

In conclusion, we demonstrated that, in ovarian adenocarcinoma cells, CD437 exerts its antitumor activity through the TBP2 pathway, where potential candidates for novel targets in drug development exist. Further studies are required to gain insight into the precise mechanisms of action of CD437 and to improve the efficacy of ovarian cancer chemotherapy.

References

1 Ho SM. Estrogen, progesterone and epithelial ovarian cancer. *Reprod Biol Endocrinol* 2003; **1**: 73.
2 Larma J, Gardner GJ. Ovarian cancer. In: Fortner KB, Szymanski LM, Fox HE, Wallach EE, eds. *The Johns Hopkins Manual of Gynecology and Obstetrics*, 3rd edn. Philadelphia: Lippincott Williams & Wilkins, 2006; 508–25.
3 Rosenthal A, Jacobs I. Ovarian cancer screening. *Semin Oncol* 1998; **25**: 315–25.
4 McGuire WP, Hoskins WJ, Brad MF. Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. *N Engl J Med* 1996; **334**: 1–6.
5 Kikkawa F, Nawa A, Ino K, Shibata K, Kajiyama H, Nomura S. Advances in treatment of epithelial ovarian cancer. *Nagoya J Med Sci* 2006; **68**: 19–26.
6 Watanabe Y, Tsuchiya H, Sakabe T *et al*. CD437 induces apoptosis in ovarian adenocarcinoma cells via ER stress signaling. *Biochem Biophys Res Commun* 2008; **366**: 840–7.
7 Gonda K, Tsuchiya H, Sakabe T *et al*. Synthetic retinoid CD437 induces mitochondria-mediated apoptosis in hepatocellular carcinoma cells. *Biochem Biophys Res Commun* 2008; **370**: 629–33.
8 Bernard BA, Bernardon JM, Delescluse C *et al*. Identification of synthetic retinoids with selectivity for human nuclear retinoic acid receptor gamma. *Biochem Biophys Res Commun* 1992; **186**: 977–83.
9 Han YH, Cao X, Lin B *et al*. Regulation of Nur77 nuclear export by c-Jun N-terminal kinase and Akt. *Oncogene* 2006; **25**: 2974–86.
10 Boisvieux-Ulrich E, Sourdeval M, Marano F. CD437, a synthetic retinoid, induces apoptosis in human respiratory epithelial cells via caspase-independent mitochondrial and caspase-8-dependent pathways both up-regulated by JNK signaling pathway. *Exp Cell Res* 2005; **307**: 76–90.
11 Cincinelli R, Dallavalle S, Merlini L *et al*. A novel atypical retinoid endowed with proapoptotic and antitumor activity. *J Med Chem* 2003; **46**: 909–12.
12 Parrella E, Giannì M, Fratelli M *et al*. Antitumor activity of the retinoid-related molecules (E)-3-(4′-hydroxy-3′-adamantylbiphenyl-4-yl) acrylic acid (ST1926) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437) in F9 teratocarcinoma: role of retinoic acid receptor gamma and retinoid-independent pathways. *Mol Pharmacol* 2006; **70**: 909–24.
13 Garattini E, Parrella E, Diomede L *et al*. ST1926, a novel and orally active retinoid-related molecule inducing apoptosis in myeloid leukemia cells: modulation of intracellular calcium homeostasis. *Blood* 2004; **103**: 194–207.
14 Langdon SP, Rabiasz GJ, Ritchie AA *et al*. Growth-inhibitory effects of the synthetic retinoid CD437 against ovarian carcinoma models *in vitro* and *in vivo*. *Cancer Chemother Pharmacol* 1998; **42**: 429–32.
15 Schadendorf D, Kern MA, Artuc M *et al*. Treatment of melanoma cells with the synthetic retinoid CD437 induces apoptosis via activation of AP-1 *in vitro*, and causes growth inhibition in xenografts *in vivo*. *J Cell Biol* 1996; **135**: 1889–98.
16 Zhang Y, Dawson MI, Mohammad R *et al*. Induction of apoptosis of human B-CLL and ALL cells by a novel retinoid and its nonretinoidal analog. *Blood* 2002; **100**: 2917–25.
17 Sun SY, Yue P, Chen X, Hong WK, Lotan R. The synthetic retinoid CD437 selectively induces apoptosis in human lung cancer cells while sparing normal human lung epithelial cells. *Cancer Res* 2002; **62**: 2430–6.
18 Hail N Jr, Lotan R. Synthetic retinoid CD437 promotes rapid apoptosis in malignant human epidermal keratinocytes and G1 arrest in their normal counterparts. *J Cell Physiol* 2001; **186**: 24–34.
19 Nishiyama A, Matsui M, Iwata S *et al*. Identification of thioredoxin-binding protein-2/vitamin D(3) up-regulated protein 1 as a negative regulator of thioredoxin function and expression. *J Biol Chem* 1999; **274**: 21645–50.

20 Kaimul AM, Nakamura H, Masutani H, Yodoi J. Thioredoxin and thioredoxin-binding protein-2 in cancer and metabolic syndrome. *Free Radic Biol Med* 2007; **43**: 861–8.
21 Butler LM, Zhou X, Xu WS *et al*. The histone deacetylase inhibitor SAHA arrests cancer cell growth, up-regulates thioredoxin-binding protein-2, and down-regulates thioredoxin. *Proc Natl Acad Sci USA* 2002; **99**: 11700–5.
22 Saitoh M, Nishitoh H, Fujii M *et al*. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J* 1998; **17**: 2596–606.
23 Ichijo H, Nishida E, Irie K *et al*. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* 1997; **275**: 90–4.
24 Nishinaka Y, Nishiyama A, Masutani H *et al*. Loss of thioredoxin-binding protein-2/vitamin D3 up-regulated protein 1 in human T-cell leukemia virus type I-dependent T-cell transformation: implications for adult T-cell leukemia leukemogenesis. *Cancer Res* 2004; **64**: 1287–92.
25 Duriez PJ, Shah GM. Cleavage of poly (ADP-ribose) polymerase: a sensitive parameter to study cell death. *Biochem Cell Biol* 1997; **75**: 337–49.
26 Kumar A, Soprano DR, Parekh HK *et al*. Cross-resistance to the synthetic retinoid CD437 in a paclitaxel-resistant human ovarian carcinoma cell line is independent of the overexpression of retinoic acid receptor-gamma. *Cancer Res* 2001; **61**: 7552–5.
27 Junn E, Han SH, Im JY *et al*. Vitamin D3 up-regulated protein 1 mediates oxidative stress via suppressing the thioredoxin function. *J Immunol* 2000; **164**: 6287–95.
28 Ludwig DL, Kotanides H, Le T, Chavkin D, Bohlen P, Witte L. Cloning, genetic characterization, and chromosomal mapping of the mouse VDUP1 gene. *Gene* 2001; **269**: 103–12.
29 Chen KS, DeLuca HF. Isolation and characterization of a novel cDNA from HL-60 cells treated with 1,25-dihydroxyvitamin D-3. *Biochim Biophys Acta* 1994; **1219**: 26–32.
30 Han SH, Jeon JH, Ju HR *et al*. VDUP1 upregulated by TGF-beta1 and 1,25-dihydorxyvitamin D3 inhibits tumor cell growth by blocking cell-cycle progression. *Oncogene* 2003; **22**: 4035–46.
31 Takahashi Y, Nagata T, Ishii Y, Ikarashi M, Ishikawa K, Asai S. Up-regulation of vitamin D3 up-regulated protein 1 gene in response to 5-fluorouracil in colon carcinoma SW620. *Oncol Rep* 2002; **9**: 75–9.
32 Billiet L, Furman C, Larigauderie G *et al*. Enhanced VDUP-1 gene expression by PPARgamma agonist induces apoptosis in human macrophage. *J Cell Physiol* 2008; **214**: 183–91.
33 Chen CL, Lin CF, Chang WT, Huang WC, Teng CF, Lin YS. Ceramide induces p38 MAPK and JNK activation through a mechanism involving a thioredoxin-interacting protein-mediated pathway. *Blood* 2008; **111**: 4365–74.
34 Wang Z, Rong YP, Malone MH, Davis MC, Zhong F, Distelhorst CW. Thioredoxin-interacting protein (txnip) is a glucocorticoid-regulated primary response gene involved in mediating glucocorticoid-induced apoptosis. *Oncogene* 2006; **25**: 1903–13.
35 Sheth SS, Bodnar JS, Ghazalpour A *et al*. Hepatocellular carcinoma in Txnip-deficient mice. *Oncogene* 2006; **25**: 3528–36.
36 Oka S, Liu W, Masutani H *et al*. Impaired fatty acid utilization in thioredoxin binding protein-2 (TBP-2)-deficient mice: a unique animal model of Reye syndrome. *FASEB J* 2006; **20**: 121–3.
37 Farhana L, Dawson MI, Leid M *et al*. Adamantyl-substituted retinoid-related molecules bind small heterodimer partner and modulate the Sin3A repressor. *Cancer Res* 2007; **67**: 318–25.
38 Seol W, Choi HS, Moore DD. An orphan nuclear hormone receptor that lacks a DNA binding domain and heterodimerizes with other receptors. *Science* 1996; **272**: 1336–9.
39 Seol W, Hanstein B, Brown M, Moore DD. Inhibition of estrogen receptor action by the orphan receptor SHP (short heterodimer partner). *Mol Endocrinol* 1998; **12**: 1551–7.
40 Nishizawa H, Yamagata K, Shimomura I *et al*. Small heterodimer partner, an orphan nuclear receptor, augments peroxisome proliferator-activated receptor gamma transactivation. *J Biol Chem* 2002; **277**: 1586–92.
41 Kim YS, Han CY, Kim SW *et al*. The orphan nuclear receptor small heterodimer partner as a novel coregulator of nuclear factor-kappa b in oxidized low density lipoprotein-treated macrophage cell line RAW 264.7. *J Biol Chem* 2001; **276**: 33736–40.

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