Anticancer effects on TACC3 by treatment of paclitaxel in HPV-18 positive cervical carcinoma cells

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Abstract. Previously, we used proteome analysis to identify transforming acidic coiled coil (TACC) 3 as a protein that is down-regulated upon paclitaxel treatment in cervical cancer cells. TACC3 mRNA and protein levels decreased after paclitaxel treatment in a time- and dose-dependent manner, and the transactivation of TACC3 promoter was dramatically diminished by paclitaxel. Importantly, paclitaxel treatment and knockdown of TACC3 by siRNA led to a synergistic enhancement of significant G2/M phase arrest and apoptosis in HeLa cells. In contrast to TACC3-deficient cells, paclitaxel treatment of mTACC3-overexpressing cells failed to induce G2/M phase arrest, cell growth inhibition, and apoptotic cell death. We studied the associated gene in mTACC over-expressed cells using microarray. From these results, numerous genes have been identified as being associated with tumor progression (Ppia, TMSB10, Annexin A2, rab31, prostaglandin E2-EP2, UHRF1), chemoresistance (Akt, Plk-1, MAP kinase) and metastasis (MMP9, PECAM-1) in mTACC3 overexpressed HeLa cells. Thus, TACC3 is thought to be the critical molecule in mediating the anticancer mechanisms of paclitaxel in p53 inactivated cells by inducing G2/M arrest and apoptosis. And our data suggested that the overexpression of TACC3 may be associated with the mechanisms of chemoresistance, tumor progression, cell proliferation and metastasis.

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Introduction

Microtubules are highly dynamic cytoskeletal fibers that play an important role in the processes of mitosis and cell division, and are a target for anticancer drugs (1). The currently most successful microtubule-targeted chemo-therapeutic agent is paclitaxel (1), which has been used to treat ovarian, breast, gastric, colorectal, lung and recurrent cervical cancer (2). Despite the relevant contribution of paclitaxel in ameliorating the quality of life and overall survival of cancer patients, drug resistance to paclitaxel represents an extremely important clinical problem. Many patients, even those who initially respond to therapy, fail to respond further during disease relapse, and a small number of patients do not even respond during the first cycle of therapy (3). Taxane, paclitaxel and docetaxel resistance is associated with multiple cellular events such as tubulin mutation (4), P-glycoprotein (Pg) overexpression (5), and increased microtubule dynamics associated with altered microtubule-associated protein (MAP) expression (6). In our previous study, we examined the anticancer mechanism of paclitaxel and analyzed the expression profiles of paclitaxel-treated HeLa cervical cancer cells by proteome analysis (2). Among the identified candidate target molecules, we focused on the characterization of the transforming acidic coiled-coil containing protein (TACC) 3. Lesperance *et al* (7) also reported using DNA microarray screening that TACC3 was one of the chemoresistant associated genes. The TACC family was originally identified as a group of three related proteins containing a highly homologous, C-terminal acidic coiled-coil domain; the respective genes localize at breast cancer amplicons and in a region disrupted in multiple myeloma (8,9). The TACC3 gene was the last of the family members to be identified in a yeast two-hybrid screen for ARNT-interacting proteins (9), and as an erythropoietin-induced gene in erythroid progenitor cells (10). TACC3 deficiency causes embryonic lethality during mid-to late gestation, which involves several lineages of cells and is associated with a high rate of apoptosis and elevated expression of the p53 target gene, p21Waf1/Cip1 (11). Thus, TACC3 is a critical component of the centrosome/mitotic spindle

apparatus, and its absence triggers p53-mediated apoptosis. Schneider *et al* (12) also reported that TACC3 depletion strongly sensitized NIH3T3 cells to paclitaxel treatment by p53-dependent induction of p21WAF and cell cycle arrest. However, it has been reported that p53 status does not affect chemosensitivity of human ovarian cancer cell lines to paclitaxel (13). In cervical cancer cells, human papillomavirus (HPV) E6 disrupt the wild-type p53 function. In this study, we functionally characterized TACC3 as a potential target of paclitaxel cytotoxicity by knockdown or overexpression of TACC3 in HPV-18 positive HeLa cells.

Materials and methods

Cell culture and cell growth measurement. HeLa cervical cancer cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Gibco BRL, Gaithersburg, MD) at 37˚C in 5% CO_2. Subconfluent proliferating cells plated in 96-well plates were treated with different doses of paclitaxel, and assayed for MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] (Sigma, St. Louis, MO) reduction, a measure of mitochondrial viability. In brief, cells were harvested from exponential phase cultures growing in DMEM supplemented with 10% FBS, counted, and plated in 96-well flat-bottomed microtiter plates ($1x10^4$ cells/ml). After 72 h of continuous exposure to paclitaxel (Taxol®, BMS, Montreal, Canada), cells were incubated with MTT substrate (20 mg/ml) for 4 h, the culture medium was removed, and DMSO was added. The optical density was measured spectrophotometrically at 550 nm. Each experiment was repeated at least 3 times.

Western blotting. Equal amounts of protein from whole cell extracts were electrophoresed by denaturing 10% SDS-PAGE. Proteins were transferred onto nitrocellulose membrane and stained with Ponceau S (Sigma) to confirm equal loading. Standard blocking and washing techniques were used, and the membranes were incubated with primary anti-TACC3 antibody (Santa Cruz Biotechnology, Santa Cruz, CA), and anti-ß-actin (Sigma) in blocking buffer, followed by detection with anti-rabbit or anti-mouse IgG (Santa Cruz Biotechnology) conjugated to horseradish peroxidase. Bands were visualized using an ECL kit (Amersham Bioscience).

RT-PCR. Total RNA was isolated from cells using the RNeasy® Mini kit (Qiagen, Germany). Reverse transcription was carried out at 50˚C for 30 min, followed by 94˚C for 2 min and a first round of PCR (30 cycles) of 95˚C for 1 min, 56˚C for 1 min and 68˚C for 1 min, with a final extension at 68˚C for 7 min using TACC3 specific primers. The mRNA levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used as an internal control to normalize for the amount of RNA.

Luciferase assay. A 109-bp fragment of the human TACC3 promoter was amplified from genomic DNA by PCR using primers containing *Xho*I and *Bam*HI restriction sites, and cloned into the pG4M-poly II luciferase vector via *Xho*I and *Bam*HI restriction enzymes. HeLa cells were transfected with

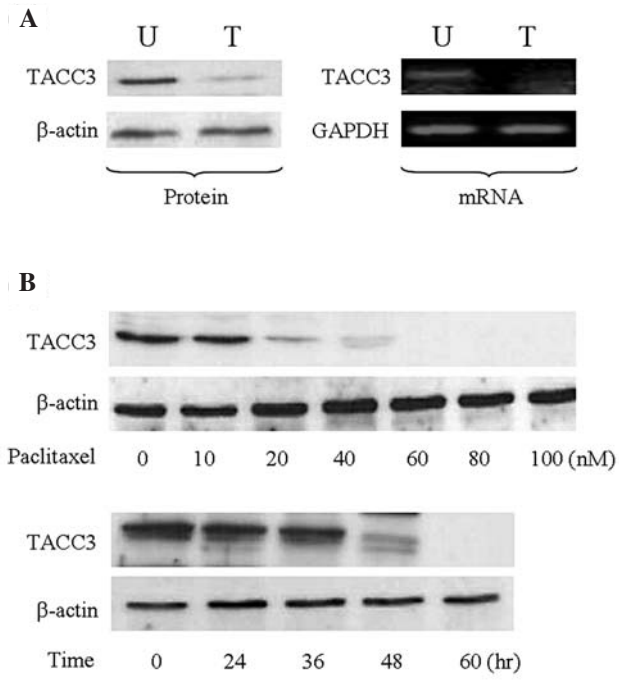


Figure 1. Effect of paclitaxel on TACC3 expression. (A) Western blotting and RT-PCR analysis of endogenous TACC3 protein and transcript levels in HeLa cells untreated (U) or treated with paclitaxel (T). ß-actin and GAPDH were used as controls. (B) Expression of TACC3 protein in HeLa cells treated with various concentrations of paclitaxel. ß-actin was used to normalize protein loading.

2 μg of either the pG4M-poly II luciferase vector driven by Gal4-fused to the TACC3 promoter sequence or the empty pG4M-poly II vector together with 0.5 μg of a pCMV-driven vector encoding ß-galactosidase. Three hours after transfection, the cells were treated with 20 nM of paclitaxel for 24 h. After removing the culture media and rinsing twice with 1X PBS, 500 μl of 1X lysis buffer was added to the plates, which were then shaken at room temperature for 15 min. The luciferase activity was determined using a luciferase assay system (Promega, Madison, WI), and the luciferase activity of each sample was normalized to ß-galactosidase activity. Each experiment was repeated at least 3 times.

Short interference RNA transfection. TACC3, and scrambled siRNAs ('Stealth RNA') were obtained from Invitrogen (Carlsbad, CA). The siRNA sequences were as follows: TACC3, 5'-CCAAAUGGUGUCUCCAGGATT-3' (sense strand) and 5'-UCCUGGAGACACCAUUUGGTT-3' (antisense strand). A scrambled siRNA (control siRNA) was synthesized for use as the control. One day prior to transfection, cells were plated in the appropriate amount of growth medium without antibiotics to ensure 30-50% confluence at the time of transfection. After 24 h, the cells were transfected with TACC3 siRNA or control siRNA at 100 pmol in serum-free medium using Lipofectamine 2000 (Invitrogen). DMEM with 10% fetal bovine serum was added after 4 h at 37˚C, and samples were incubated for another 48 h. After 48 h, total mRNA and protein lysates were harvested from cells and analyzed by RT-PCR and Western blotting.

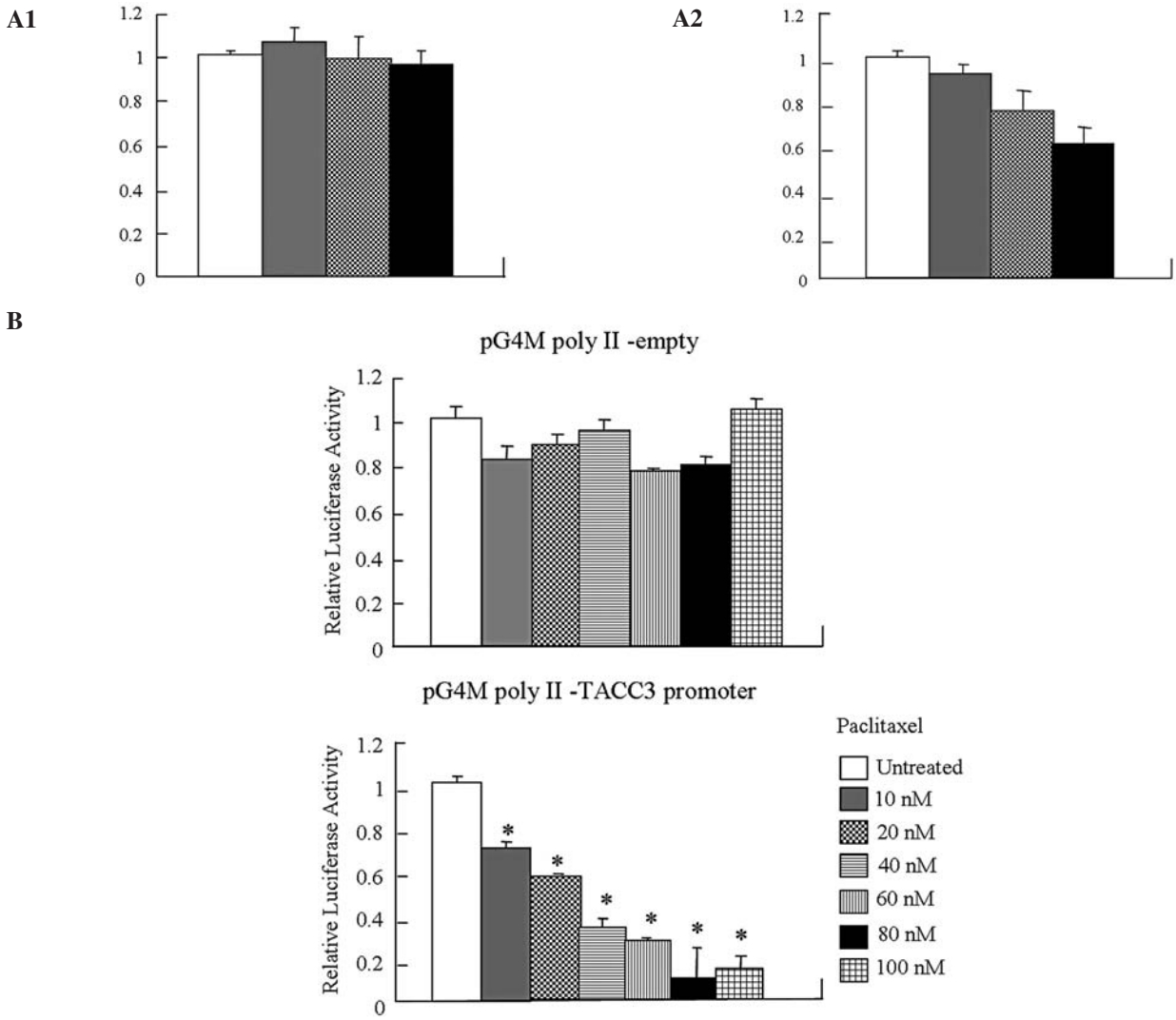


Figure 2. Paclitaxel decreased luciferase activity from construct containing TACC3 promoter. The luciferase control plasmid or the construct driven by the TACC3 promoter was transiently transfected into HeLa cells along with the ß-galactosidase reporter plasmid as an internal control. (A) Transfected cells were treated with 20 nM paclitaxel and assessed for luciferase activity after various periods of time, ranging from 0 to 24 h. (B) Cells were transfected and treated with paclitaxel at various concentrations as indicated, and the luciferase activity was assessed after 48 h. Activity was normalized to ß-galactosidase activity to determine the relative luciferase activity. These experiments were performed 4 times. $^{*}p<0.05$.

FACS analysis. For cell cycle analysis, cells were treated with 20 nM of paclitaxel for 24 h. Cells were washed in 1X PBS and fixed for 1 h in 70% ethanol at 4˚C. After treatment with 50 μg/ml RNase (Sigma) for 20 min at room temperature, cells were washed with PBS and incubated with 40 μg/ml propidium iodide (PI) (Sigma) for 30 min. All samples were analyzed using a FACS System (FACSCalibur, Becton Dickinson, Mountain View, CA).

Analysis of chromatin condensation and segregation. Paclitaxel-treated cells were harvested, washed in 1X PBS, fixed with 4% paraformaldehyde, and stained for 5 min in 0.1 mg of DAPI (Sigma) in methanol. Treated cells were analyzed via fluorescence microscopy to assess chromatin condensation and segregation.

Establishment of a stable TACC3 cell line. The cDNA sequences of mTACC3 were deposited in the Genbank database (accession no. AF093542). mTACC3 cDNA was subcloned into the pcDNA3-HA vector downstream of the cytomegalovirus (CMV) promoter. To generate stable TACC3 transformants, HeLa cells were transfected with pcDNA3-HA-mTACC3 using Lipofectamine plus reagent (Invitrogen), and cells were cultured by selection in medium containing 2 mg/ml neomycin (G418, Gibco BRL). pcDNA3-HA empty vector was transfected in HeLa cells as a control.

Microarray analysis. cDNA microarray chips containing 14K mouse genes (known gene: 9,052) supplied by Digital Genomics (Seoul, Korea) were used. Each hybridization reaction was started with 15 μg total RNA. Cy3/Cy5 fluorescent dye (Amersham Pharmacia, Piscataway, NJ) labeling and hybridization were carried out by Digital Genomics. Signals hybridized with Cy3- and Cy5-labeled probes were read at 532 and 635 nm by using ScanArray 3.0 (Packard). Image data were analyzed using GenePix Pro 4.0 (Axon Instruments, Burlingame, CA).

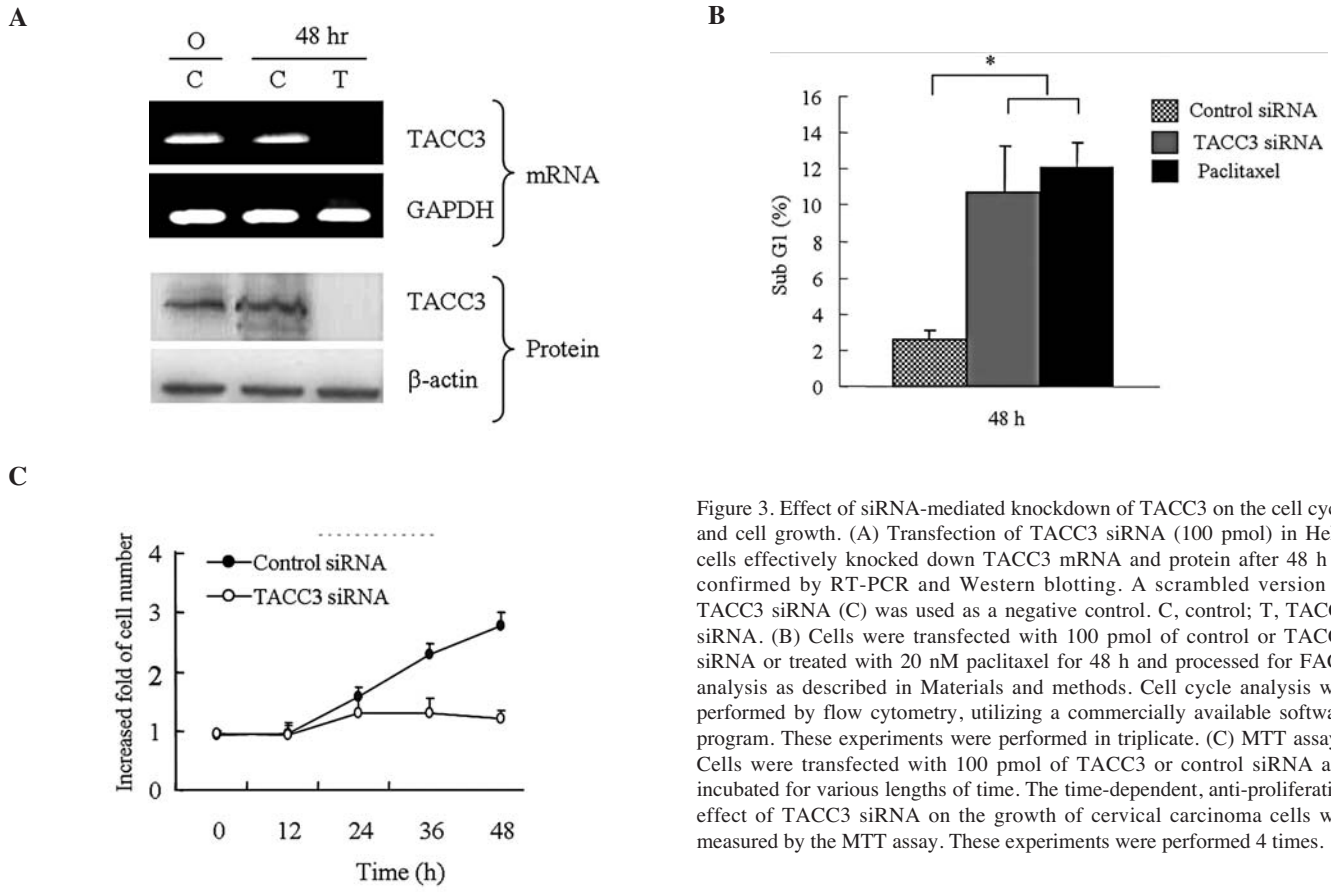


Figure 3. Effect of siRNA-mediated knockdown of TACC3 on the cell cycle and cell growth. (A) Transfection of TACC3 siRNA (100 pmol) in HeLa cells effectively knocked down TACC3 mRNA and protein after 48 h as confirmed by RT-PCR and Western blotting. A scrambled version of TACC3 siRNA (C) was used as a negative control. C, control; T, TACC3 siRNA. (B) Cells were transfected with 100 pmol of control or TACC3 siRNA or treated with 20 nM paclitaxel for 48 h and processed for FACS analysis as described in Materials and methods. Cell cycle analysis was performed by flow cytometry, utilizing a commercially available software program. These experiments were performed in triplicate. (C) MTT assays. Cells were transfected with 100 pmol of TACC3 or control siRNA and incubated for various lengths of time. The time-dependent, anti-proliferative effect of TACC3 siRNA on the growth of cervical carcinoma cells was measured by the MTT assay. These experiments were performed 4 times.

Statistical analysis. All observations were confirmed by at least three independent experiments. The Student's t-test was used to evaluate the statistical significance of mean values. Values of $p < 0.05$ were considered to be significant.

Results

Paclitaxel down-regulated transcriptional activity of TACC3 promoter and TACC3 expression in HeLa cells. The cell survival rate was significantly reduced by paclitaxel treatment in a dose- and time-dependent manner. The IC_{50} value of paclitaxel was determined by interpolation from the curves, and found to be 20 nM for HeLa cells.

To assess the effects of paclitaxel on TACC3 protein and mRNA levels, cells were harvested after 48 h of treatment with paclitaxel, and their total lysates and mRNA were analyzed by Western blotting and RT-PCR. As shown in Fig. 1A, dramatic suppression of both TACC3 mRNA and protein expression was observed in response to paclitaxel. Furthermore, the partial to complete suppression of TACC3 protein levels occurred in a time- and dose-dependent manner (Fig. 1B). Based on our data that paclitaxel decreased TACC3 mRNA levels, we next used a transient *TACC3* promoter luciferase reporter assay to evaluate whether paclitaxel could decrease *TACC3* promoter activity in HeLa cells. Upon exposure of HeLa cells transfected with the pG4M poly II luciferase vector driven by TACC3 promoter to 20 nM paclitaxel, we observed a marked and time-dependent decrease in luciferase activity (Fig. 2A). We also found that paclitaxel treatment diminished *TACC3* promoter activity in a dose-dependent manner (Fig. 2B). Together these results indicate that paclitaxel exerts inhibitory effects on *TACC3* gene transcription.

Cell cycle and cell growth effects in TACC3 siRNA treated HeLa cells. To address whether *TACC3* could be a suitable pharmacological target for paclitaxel cytotoxicity, we first employed the RNA interference (RNAi) system in cultured HeLa cells and confirmed a dramatic decrease in TACC3 mRNA transcript and protein levels (Fig. 3A). We analyzed the cell cycle distribution after transfection of *TACC3* siRNA in HeLa cells. As shown in Fig. 3B, we observed an increase in the G2/M population with a concomitant decrease in the G0/G1 population after *TACC3* siRNA treatment in HeLa cells. Moreover, after 48 h, we observed an obvious and significant increase in the sub-G1 population. We also observed that the effect of knockdown of *TACC3* on the cell cycle was similar to that of paclitaxel. We investigated the effects of *TACC3* siRNA on the growth of HeLa cells. HeLa cell proliferation was significantly suppressed by *TACC3* siRNA compared with control siRNA (Fig. 3C).

Together these results indicated that RNAi-mediated specific knockdown of *TACC3* induced abnormal accumulation of cells in the G2/M phase, strong inhibition of cell growth *in vitro*, and apoptosis.

Knockdown of TACC3 significantly enhances the cytotoxicity of paclitaxel. Based on the ability of RNAi-mediated knockdown of TACC3 to arrest cells in G2/M phase, we speculated that it could synergistically enhance the cytotoxicity

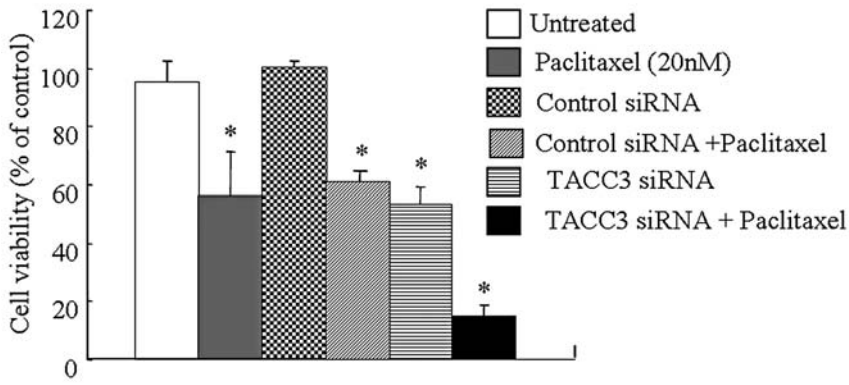
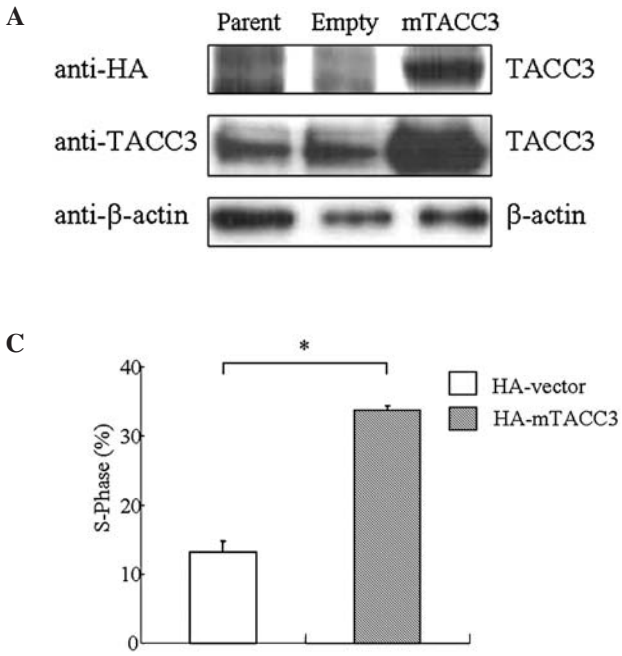


Figure 4. Synergistic effect of knockdown of TACC3 and paclitaxel treatment on cytotoxicity. Viable HeLa cells quantified by MTT assay after transfection with control or TACC3 siRNA, treatment with 20 nM of paclitaxel, or a combination of the two. These experiments were performed 4 times. *p<0.05.



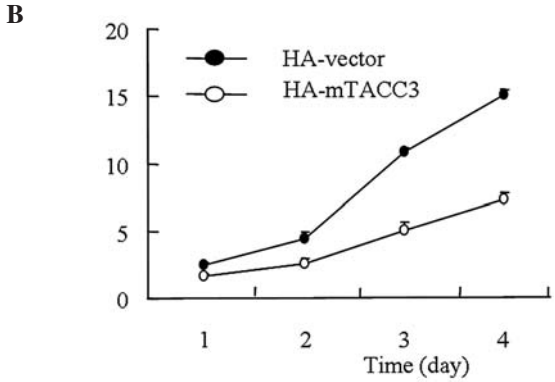


Figure 5. Establishment and characterization of the mTACC3-overexpressing stable cell line. (A) Western blot analysis for HA, TACC3, and ß-actin in untransfected HeLa cells (parent), HeLa cells overexpressing the empty HA vector (empty), and HA-mTACC3-overexpressing HeLa cells (mTACC3). (B) Cell growth analysis by MTT assay of HA-vector and HA-mTACC3 overexpressing HeLa cells over 5 days. These experiments were performed 4 times. (C) FACS analysis of HA-vector and HA-mTACC3 stable cell lines. Total amount of cells in S-phase was determined by flow cytometry. These experiments were performed 4 times. *p<0.05.

of paclitaxel. To assess this possibility, we investigated the synergistic effects of TACC3 siRNA and paclitaxel in HeLa cell lines. The concentration of paclitaxel was set by the IC_{50} of HeLa cells, as previously determined by the MTT assay. We then used the MTT assay to check the viability of cells treated with control siRNA, TACC3 siRNA, paclitaxel, or a combination of paclitaxel and siRNA treatment. Although single treatment with TACC3 siRNA or paclitaxel induced a significant cytotoxic effect, a synergistic enhancement of the cytotoxic effect of paclitaxel by TACC3 siRNA was observed in HeLa cells (Fig. 4). Taken together, these results indicate that RNAi-mediated specific knock-down of TACC3 can synergistically enhance the chemosensitivity of cells to paclitaxel.

Resistance of TACC3-overexpressing cells to paclitaxel. We wondered whether the overexpression of TACC3 would affect cancer cell resistance to paclitaxel cytotoxicity *in vitro*. To address this question, we established a stable transfectant HeLa cell line expressing mouse TACC3 (mTACC3). The mTACC3-overexpressing HeLa cell line showed elevated expression of the exogenous TACC3 protein (Fig. 5A). mTACC3-overexpressing HeLa cells showed cell overgrowth (Fig. 5B) and S-phase delay (Fig. 5C) as compared with control cells. We next determined whether the over-expression of TACC3 would affect cancer cell resistance against paclitaxel cytotoxicity *in vitro*. We analyzed the cell cycle distribution after paclitaxel treatment of mTACC3-overexpressing HeLa cells. As shown in Fig. 6A, compared to the pronounced effect of paclitaxel on G2/M and sub-G1 accumulation in control cells, no change was observed in cell cycle distribution upon paclitaxel treatment of cells over-expressing mTACC3. Moreover, unlike the morphological changes observed in control cells treated with paclitaxel,

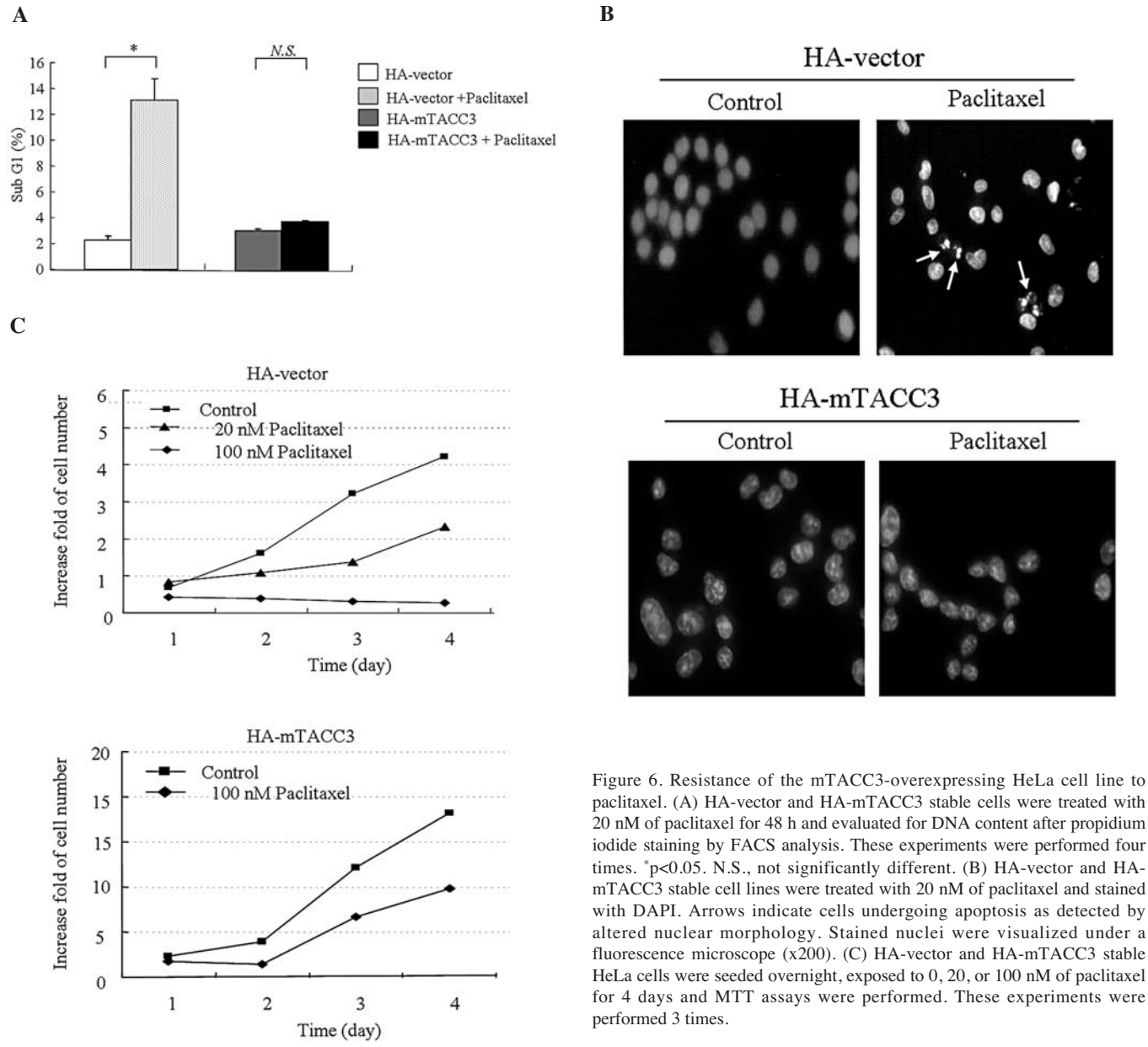


Figure 6. Resistance of the mTACC3-overexpressing HeLa cell line to paclitaxel. (A) HA-vector and HA-mTACC3 stable cells were treated with 20 nM of paclitaxel for 48 h and evaluated for DNA content after propidium iodide staining by FACS analysis. These experiments were performed four times. $^{*}p<0.05$. N.S., not significantly different. (B) HA-vector and HA-mTACC3 stable cell lines were treated with 20 nM of paclitaxel and stained with DAPI. Arrows indicate cells undergoing apoptosis as detected by altered nuclear morphology. Stained nuclei were visualized under a fluorescence microscope (x200). (C) HA-vector and HA-mTACC3 stable HeLa cells were seeded overnight, exposed to 0, 20, or 100 nM of paclitaxel for 4 days and MTT assays were performed. These experiments were performed 3 times.

mTACC3-overexpressing HeLa cells did not show any increase of cells with apoptotic morphology (Fig. 6B). Cellular proliferation was evaluated by the MTT assay daily for 4 days, and treatment with 20 nM paclitaxel had no effect on the proliferation of mTACC3-overexpressing HeLa cells. Even at paclitaxel concentrations over 3 times the IC_{50} value, only a slight decrease in cell growth was observed (Fig. 6C). These results indicate that stable expression of mTACC3 is strongly associated with resistance to paclitaxel treatment.

Gene expression changes by mTACC3 overexpression in HeLa cells. Table I showed list of selected genes that were up-regulated by mTACC3 overexpression. There is several up-regulated genes with previously reported in mechanisms of chemoresistance [Akt (14), Plk-1 (15), MAP kinase (16)], tumor progression and proliferation [Ppia (7), TMSB10 (17), Annexin A2 (18), rab31 (19), prostaglandin E2-EP2 (20), UHRF1 (21)], and metastasis associated molecules [MMP9 (22,23), PECAM-1 (24)].

Table II shows a list of selected genes that were down-regulated by mTACC3 overexpression. This list includes genes that are implicated in chemosensitivity [caspase-3(25), E2F-4(26), IGF1R(27)], cell cycle regulation (ubiquitine-activating enzyme E1C, Gspt2, Itgb1, calcium/calmodulin-dependent protein kinase II γ, growth arrest and DNA-damage-inducible 45 α), and apoptosis (caspase 3, cell division cycle and apoptosis regulator 1, traf4).

Discussion

Paclitaxel-induced cytotoxicity is associated directly with mitotic arrest (28) or following an aberrant mitotic exit (28,29). However, the mechanisms downstream of the stabilization of microtubule dynamics leading to apoptosis remain unclear, and the mitotic spindle assembly check-

Table I. Genes up-regulated by mTACC3 overexpression.

Functional category	GenBank accession no.	Gene name
Apoptosis	NM_009652	Thymoma viral proto-oncogene 1 (Akt1)
Cell cycle	AK076351	Extra spindle poles-like 1
	NM_011121	Polo-like kinase 1 (*Drosophila*)
	BC023116	Cell growth regulator with EF hand domain 1
Cellular physiology	NM_011295	Ribosomal protein S12
	NM_008907	Peptidylprolyl isomerase A (Ppia)
	NM_021278	Thymosin, ß4, X chromosome
	NM_172086	Ribosomal protein L32
	NM_013798	Actin, γ, cytoplasmic 1
	NM_008503	Phosphodiesterase 6A, cGMP-specific, rod, α (Pde6a)
	NM_016682	Ubiquitin-like 1 (sentrin) activating enzyme E1B (Uble1b)
	NM_010448	Heterogeneous nuclear ribonucleoprotein A/B (Hnrpab)
	NM_025284	Thymosin, ß10 (Tmsb10)
	NM_010880	Nucleolin
	NM_007585	Annexin A2
	NM_019472	Myosin X
	NM_013599	Matrix metallopeptidase 9 (Mmp9)
Immune response	NM_133212	Toll-like receptor 8 (Tlr8)
Response to stress	NM_008302	Heat shock protein 1, ß (Hspcb)
Signal transduction	NM_009062	Regulator of G-protein signaling 4
	NM_146592	Olfactory receptor 1086 (Olfr1086)
	AK043482	MAP Kinase Kinase
	NM_008816	Platelet/endothelial cell adhesion molecule 1, transcript variant 2
	NM_008964	Prostaglandin E receptor 2 (subtype EP2)
	NM_133685	RAB31, member RAS oncogene family (Rab31)
Transcription	BU611745	RIKEN cDNA A630018P17 gene (A630018P17Rik)
	NM_010710	LIM-homeodomain protein MLHX2 (Lhx2)
	NM_010931	Ubiquitin-like, containing PHD and RING finger domains, 1
	AF448482	Homeo box C12 (Hoxc12)

point has been proposed to regulate paclitaxel-initiated apoptosis (30). TACC3, one of the important structural components of the centrosome/spindle apparatus (31). stabilizes centrosomal microtubules and is required to recruit ch-TOG/XMAP215/Msps to the centrosome in an Aurora-A regulated manner (32,33). This complex is critical to maintain centrosome integrity, centrosome-dependent assembly of microtubules and spindle stability (34,35).

Our results showed that paclitaxel diminished TACC3 transcription in a time- and dose-dependent manner. TACC3 knockdown only was a similar change to that of paclitaxel treatment in HeLa cells. Both paclitaxel treatment and RNAi-mediated knockdown of TACC3 induced significant G2/M phase arrest, strong suppression of growth, and eventual cell apoptosis. In contrast to TACC3-deficient cells, paclitaxel treatment failed to induce G2/M phase arrest, cell growth inhibition, or apoptosis in mTACC3-overexpressing HeLa cells. Interestingly, minimal cytotoxicity was observed at a concentration of paclitaxel that was >3 times the IC_{50}. These results indicate that stable expression of mTACC3 is associated with resistance to paclitaxel treatment.

Consistent with the results from Schneider *et al* (12) we found a synergistic enhancement of paclitaxel cytotoxicity after RNAi-mediated knockdown of TACC3 in HeLa cells. However, they discussed that TACC3-depleted cells had different effects on cell cycle progression depending on the presence or absence of functional p53 (12). Paclitaxel induced p53- and p21-dependent arrest in postmitotic G1 or G2 arrest in cell type-dependent fashion (12,36). However, most cancer cells including HeLa cells had the mutated or inactivated p53 gene (37). Unlike postmitotic arrest, mitotic arrest and apoptosis are p53-independent (28,38,39). In these reports, both paclitaxel treatment and knockdown of TACC3 induced significant G2/M phase arrest and apoptosis. In our previous study, we observed dual apoptotic pathways by paclitaxel treatment in HeLa cells, through the membrane

Table II. Genes down-regulated by mTACC3 overexpression.

Functional category	GenBank accession no.	Gene name
Cell cycle	NM_011666	Ubiquitin-activating enzyme E1C
	NM_008179	G1 to S phase transition 2 (Gspt2)
	AK088016	Integrin ß1 (fibronectin receptor ß) (Itgb1)
	AK078311	Calcium/calmodulin-dependent protein kinase II γ
	NM_007836	Growth arrest and DNA-damage-inducible 45 α
Apoptosis	NM_009810	Caspase 3
	AF465615	Cell division cycle and apoptosis regulator 1
	NM_009423	TNF receptor associated factor 4 (traf4)
Transcription	NM_139117	Cold shock domain protein A (Csda)
	NM_148952	E2F transcription factor 4
	AK088142	Minichromosome maintenance deficient 3)
	NM_008229	Histone deacetylase 2 (Hdac2)
	AK088142	Minichromosome maintenance deficient 3
	NM_008186	General transcription factor II H, polypeptide 1 (Gtf2h1)
Cellular physiology	NM_172394	Nucleoporin 88
	NM_007830	Diazepam binding inhibitor
	NM_025859	ADP-ribosylation factor-like 1 (Arl1)
	NM_016754	Myosin light chain, phosphorylatable, fast skeletal muscle
	NM_009128	Stearoyl-Coenzyme A desaturase 2 (Scd2)
Response to stress	NM_009825	Serine (or cysteine) peptidase inhibitor, clade H, member 1
	NM_133804	Heat shock 70kDa protein 5 binding protein 1 (Hspa5bp1)
	NM_008303	Heat shock protein 1 (chaperonin 10)
	NM_028450	GULP, engulfment adaptor PTB domain containing 1
	NM_011363	PSM/SH2-B delta (Sh2bpsm1)
	NM_029576	RAB1B, member RAS oncogene family (Rab1b)
	BC033382	RAS related protein 1b
	NM_016719	Growth factor receptor bound protein 14
	AK087668	Insulin-like growth factor I receptor (Igf1r)
	NM_008543	MAD homolog 7 (*Drosophila*) (Smad7)
	NM_007982	PTK2 protein tyrosine kinase 2
	AK088016	Integrin ß1 (fibronectin receptor ß) (Itgb1)

death receptor (TRAIL)-mediated apoptotic pathway and cytochrome-c-dependent (bcl-2-mediated) apoptotic pathway (2). Recent studies demonstrated that Akt activation plays a significant role in protection of TRAIL-induced apoptosis and Akt dephosphorypation induced by paclitaxel correlated with TRAIL sensitization (40). We studied the associated gene in mTACC overexpressed cells using microarray. From these results, numerous genes have been identified as being associated with tumor progression and chemoresistance in mTACC3 overexpressed HeLa cells. Akt was also up-regulated having shown chemoresistance phenotype through its antiapoptotic activity (14). A recent study (15) showed Plk-1 association with gencitabine chemosensitivity. The MAP kinase showed up-regulation in these studies, consistent with previous reports suggesting a relationship between MAPK gene expression and paclitaxel cytotoxicity (16). On the other hand, some genes involved in chemosensitivity [caspase-3 (25), E2F-4 (26), IGF1R (27)] were observed to be down-regulated in mTACC3 overexpressed HeLa cells.

In summary, TACC3 is thought to be the critical molecule in mediating the anticancer mechanisms of paclitaxel in p53 inactivated cells by inducing G2/M arrest and apoptosis. Our data suggest that the overexpression of TACC3 may be associated with the mechanisms of chemoresistance, tumor progression, cell proliferation and metastasis. Although further *in vivo* validations for the identified genes are required because the materials used in the study were induced cells *in vitro*, this important information may lead to the discovery of new paclitaxel resistance targets. Further detailed investigation is needed to understand the mutual relationship and molecular function of TACC3 for paclitaxel treatment in cervical carcinoma cells.

Acknowledgements

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