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Identification of a novel role of Septin 10 in paclitaxel-resistance in cancers through a functional genomics screen

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Paclitaxel (also known as taxol) is a member of the taxane class of anticancer agents, which has a well-known mechanism that blocks cell mitosis and kills tumor cells, that is often used in clinics to treat cancer. However, some carcinomas such as breast, ovarian and non-small-cell lung cancers are often resistant to paclitaxel treatment. In this study, we used a lentiviral siRNA library against the entire human genomes to identify genes associated with sensitivity to paclitaxel. We isolated two paclitaxel-resistant clones carrying the siRNA specific to *septin 10* (*SEPT10*) and to budding uninhibited by benzimidazoles 3. The relation of budding uninhibited by benzimidazoles 3 to paclitaxel sensitivity has already been established, but that of *SEPT10* remains unknown. Interestingly, overexpression of SEPT10 increased cells' sensitivity to paclitaxel; we also found that SEPT10 is an important regulator for microtubule stability. Furthermore, we found that paclitaxel-resistant tumors had decreased expression of SEPT10. Thus, SEPT10 may be a novel candidate molecule that acts as a good indicator of paclitaxel-resistant carcinomas (*Cancer Sci* 2012; 103: 821–827)

Therapeutic interference with the mitotic spindle apparatus is now a widely applied rationale for the treatment of various malignant tumors. Paclitaxel, a prototype taxane, is one of the most effective chemotherapy drugs for the treatment of many solid tumors including ovarian, breast, prostate and non-small-cell lung cancers. It works by binding tubulin and stabilizing nonfunctional microtubule bundles, thereby blocking normal mitotic spindle development and subsequent cell division.[1] Its clinical indications continue to expand. However, paclitaxel resistance limits clinical utility to approximately 50% of patients with breast and ovarian cancers. Therefore, it is necessary to identify the mechanism and therapeutically accessible molecular markers for paclitaxel resistance to improve cancer treatments. The main mechanisms for paclitaxel resistance include defective intracellular drug accumulation mediated by overexpression of the drug transporter P-glycoprotein,[2] as well as microtubule-related mechanisms such as mutations in the β-tubulin gene,[3,4] differential expression of β-tubulin isotypes,[5] altered microtubule dynamics,[6] and deregulation of the mitosis regulator and inhibitor of apoptosis protein surviving.[7] Furthermore, we recently revealed that USP15 plays an essential role in the stability and activity of caspase-3 during paclitaxel-induced apoptosis and that knockdown of USP15 in HeLa cells causes paclitaxel resistance.[8]

Septins are a family of conserved GTP-binding proteins that act as dynamic, regulatable scaffolds for the recruitment of other proteins.[9–13] Septins were originally identified from yeast, with cytokinesis mutants as the molecules involved in many processes including membrane dynamics, vesicle trafficking, apoptosis, infection and cytoskeletal remodeling.[10–12,14] Recently, it has been reported that mammalian septins modulate microtubule dynamics through the interaction with a microtubule-associated protein, MAP4.[15] In addition, *SEPT10* has been cloned as a novel septin family member and has shown to be ubiquitously expressed in the normal tissues.[16] However, the molecular functions of SEPT10 are still not completely understood.

Using RNA interference as a tool for gene silencing in mammalian cells has opened up the possibility of performing high-throughput loss-of-function screens in tissue culture systems.[17–20] We screened genes associated with paclitaxel sensitivity with the GeneNet Human 50K siRNA Library (System Biosciences, Mountain View, CA, USA) and HeLa cells, and have obtained a few genes. We have previously reported that one of the genes is *USP15*.[8] In this study, we have identified two additional genes, *BUB3* and *SEPT10*, that are associated with paclitaxel sensitivity. The relation of *BUB3* to paclitaxel-induced cell cycle arrest has been previously reported,[21] but relations between *SEPT10* and paclitaxel sensitivity remain unknown. Here, we reveal that SEPT10 plays an essential role in paclitaxel-induced cell apoptosis through controlling microtubules. Furthermore, ovarian cancer specimens resistant to paclitaxel treatment showed decreased expression of SEPT10 and caspase-3 protein relative to paclitaxel-sensitive specimens. Thus, the hallmark of the *SEPT10* gene may be important in cancer therapies for some types of paclitaxel-resistant cancers.

Materials and Methods

High-throughput screening of the siRNA that affect paclitaxel resistance. The screening system using a lentiviral siRNA library was previously described.[8] Briefly, the GeneNet Human 50K siRNA Library was cloned into an FIV-based pFIV-H1 shRNA expression vector. For screening, HeLa cells were infected with the shRNA viral particles. After 5 days, the infected library cells were selected with 2-μg/mL puromycin. The selected cells were treated with 10-nM paclitaxel (Wako Pure Chemical Industries, Osaka, Japan) for 48 h and continued to be cultured without paclitaxel for 10–14 days. When paclitaxel-resistant colonies arose, the nucleotide sequence of integrated shRNA in each paclitaxel-resistant colony was determined.

Cell culture and siRNA transfection. Both HeLa and Michigan Cancer Foundation (MCF)-7 cells were grown in DMEM

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(Sigma-Aldrich, St. Louis, MO, USA) containing 10% FBS at 37°C in a 5% CO_2 environment. Cell numbers were quantitated with an erythrometer. The results are representative of three independent cell count analyses.

For transient siRNA transfection, HeLa cells were transfected with 250-nM chemically synthesized double-stranded siRNA (siTrio) specifically for *BUB3* (1712-1, GAACACUGAUCAAGAAAAUdTdT; 1712-2, GCAGAGUGUUGGUGUGGGAdTdT; 1712-3, GCCCUGAGGUACAGAAGAAdTdT) and for *SEPT10* (1479-1, GGAAACUGGAAUUGGAAAAdTdT; 1479-2, GCUGCAAACUGGAGGAAAUdTdT; 1479-3, GAGAAUGAAGCUUGAAGAAdTdT) (B-Bridge International, Cupertino, CA, USA). Then, 12-μL HiPerFect reagent (Qiagen K. K., Tokyo, Japan) was added to each 60-mm culture dish according to the manufacturer's recommendations. Non-silencing control siRNA (AUCCGCGCGAUAGUACGUAdTdT) (B-Bridge International) was also transfected as a negative control.

Generation of a stable SEPT10-expressed cell clone. The cDNA fragment that corresponds to the complete coding region of *SEPT10* was amplified by RT-PCR from HeLa cells with the primers, 5′-CACCATGGCCTCCTCCGAGGTGGC-3′ and 5′-CAAAAAATTGGAGTTCTTAC-3′. The amplified cDNA was cloned into the pcDNA-DEST40 expression vector (pDEST40). To generate a stable SEPT10-expressed clone, the SEPT10 expression vector (SEPT10-pDEST40) was transfected into MCF-7 cells with FuGENE HD transfection reagent (Roche Diagnostics, Indianapolis, IN, USA). As a negative control clone, parental pDEST40 vector was also transfected. Then, the transfected cells were cultured in the presence of 400-μg/mL Geneticin (G)418 (Calbiochem, Merck KGaA, Darmstadt, Germany), and the G418 resistant clones were isolated.

Western blot analysis. Western blot analysis was performed as described previously.[8] Antibodies anti-SEPT10 (M01, clone 2A12; Abnova, Taipei City, Taiwan), anti-BUB3 (BD Transduction Laboratories, Franklin Lakes, NJ, USA), anti-caspase-3 (Cell Signaling Technology, Danvers, MA, USA), anti-MAP4 (Novus Biologicals, Littleton, CO, USA) and anti-GAPDH (6C5; Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used at a 1:1000 dilution.

Nocodazole stability assays. Cells were treated with 10-μM nocodazole (Sigma-Aldrich) for 30 min. Then, the cells were fixed with 4% paraformaldehyde for 10 min. After the cells were washed with PBS, they were incubated with anti β-tubulin antibody (clone B-5-1-2, Sigma-Aldrich) at a 1:100 dilution in 0.02% Tween-20 in PBS for 2 h at room temperature. After the cells were washed with PBS, they were incubated with goat anti-mouse IgG (H+L) antibody conjugated with Alexa Fluor (Molecular Probes, Invitrogen, Carlsbad, CA, USA) at a 1:500 dilution in 0.02% Tween-20 in PBS, and they were then examined with an inverted fluorescence microscope (BX50; Olympus, Tokyo, Japan).

Cell cycle analysis. To determine cell cycle profiles, cells were harvested, washed, and fixed with 70% ethanol in PBS. After overnight incubation at 4°C, cells were resuspended in the staining buffer (50-μg/mL propidium iodide, 0.1% RNaseA, 1-mg/mL glucose in PBS). Then, after incubation for 30 min at room temperature, the cells were analyzed with a FACS Vantage Flow Cytometer (BD Biosciences, Franklin Lakes, NJ, USA) using the CellQuest acquisition and analysis program (BD Biosciences).

Immunoprecipitation. We treated 1×10^7 cells of parental HeLa, vehicle (pFIV-H1)-HeLa and SEPT10-kockdown-HeLa with 10-nM paclitaxel for 0 and 24 h, and at each time point, the cells were harvested and lysed in 1-mL IP buffer (0.05-M Tris–HCl [pH 7.9], 0.15-M NaCl, 50-mM EDTA, 0.05% NP-40). The sample lysate was mixed with 5.0-μg/mL anti-MAP4 antibody and incubated at 4°C overnight. Then, 20-μL 50% (v/v) Protein G Sepharose beads (GE Healthcare UK Ltd, Buckinghamshire, UK) were added to the reaction mixture, and the sample was incubated and rotated for 1 h at 4°C. After centrifugation, the supernatant was collected, and the beads were washed with IP buffer three times. Then, immunoprecipitates on the beads were subjected to Western blot analysis. Rabbit IgG and mouse IgG immunoprecipitates were used as negative controls.

Immunohistochemistry. Immunohistochemical assays were performed on formalin-fixed paraffin-embedded sections using

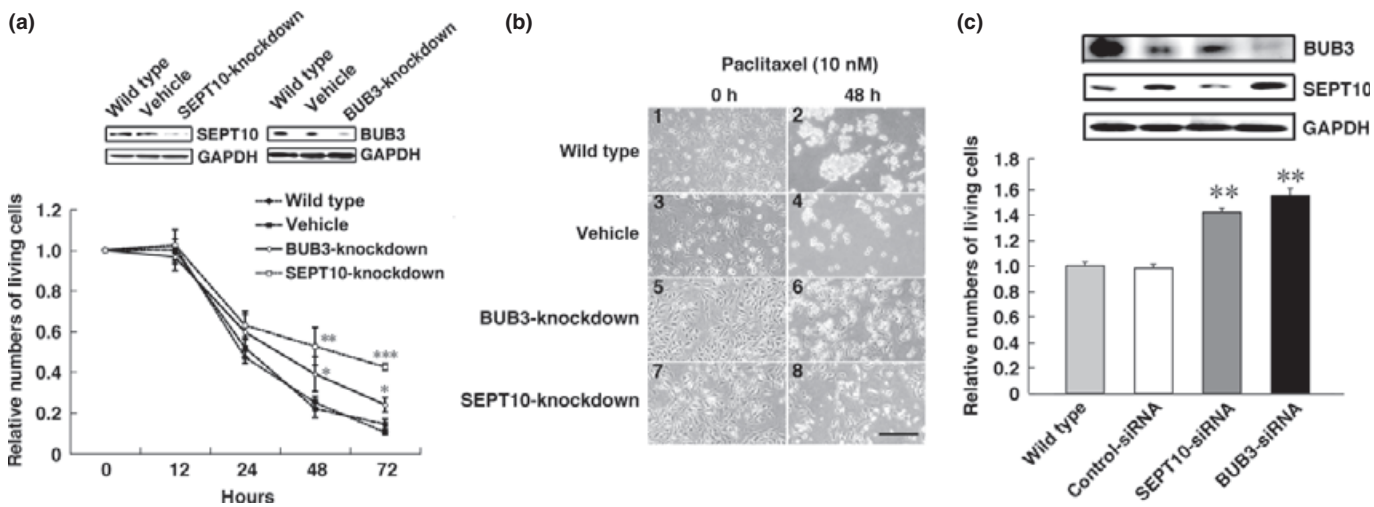

Fig. 1. Knockdown of SEPT10 or BUB3 reduces the paclitaxel sensitivity of HeLa cells. (a) The SEPT10- and BUB3-knockdowned HeLa clones had an increased number of surviving cells compared with untreated HeLa (wild-type) clones and a negative control vector (pFIV-H1)-transfected stable HeLa clone (vehicle) after paclitaxel treatment. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. (b) Representative microscopic images of the cells at 0 and 48 h after the addition of paclitaxel. Scale bar, 100 μm. (c) Western blotting confirmed the expression levels of SEPT10 and BUB3, respectively. GAPDH is shown as a loading control. HeLa cells transfected with the mixture of siRNA (siTrio) specific for SEPT10 or BUB3 also show paclitaxel resistance relative to wild-type HeLa and negative control-siRNA-transfected HeLa (**$P < 0.01$). Note that the target sites of the siTrios for *SEPT10* and *BUB3* are different from that of lentiviral shRNA in the stable clones. Cells were transfected with siRNA, and the next day paclitaxel was added to the culture medium. Then, the living cells treated with paclitaxel for 48 h were counted and subjected to Western blotting. BUB3, budding uninhibited by benzimidazoles 3; FIV, feline immunodeficiency virus; SEPT10, septin 10.

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Ventana HX System Benchmark (Tucson, AZ, USA). Mouse monoclonal anti-SEPT10 and rabbit polyclonal anti-caspase-3 antibodies (Cell Signaling Technology) were diluted at 1:50 and incubated at 37°C in a humidified chamber for 1 h. The frequency of immunostainpositive cells per 500 tumor cells was quantified.

Clinical samples. We obtained human ovarian cancer samples from the patients who had received only paclitaxel treatments for a long time, but the treatment was not effective and cancer recurred. All patients were from the Tokyo Medical University Hospital (Tokyo, Japan) and provided written informed consent. The study was approved by the institutional review board at the hospital.

Results

Screening paclitaxel-resistant colonies. To isolate molecules concerned with the acquisition of paclitaxel resistance, we used a genome-wide lentiviral siRNA library, the GeneNet Human 50K siRNA Library, that consists of 200 000 sequences of siRNA that target 47 400 human mRNA. For most of the target genes, there are four different siRNA sequences. This library has been cloned into pFIV-H1 shRNA expression vectors. After infection and puromycin selection of HeLa cells, we treated the cells with 10-nM paclitaxel for 48 h. Then, after further incubation of the cells for 10–14 days without paclitaxel, we obtained a few colonies resistant to paclitaxel. To identify the clones' siRNA-targeted mRNA, we performed nested PCR analysis with genomic DNA of the clones and used specific primers against pFIV-H1 vector; we also determined the nucleotide sequences of the PCR products. From the determined sequences, 5′-ATAAAGTACCTTTGAGCATGAGTGTAT-3′ and 5′-AGAAATGGCATTGACTGGGTAAATCTG-3′, we found that these PCR products are derived from the *SEPT10* and *BUB3* genes, respectively. Although it has been established that knockdown of *BUB3* inhibits the effects of paclitaxel on cell cycle progression,[21] *SEPT10* is novel for paclitaxel resistance.

Knockdown of *SEPT10* and *BUB3* induces paclitaxel resistance. To confirm the effects of *SEPT10* or *BUB3* knockdown on paclitaxel sensitivity, we treated the specific shRNA-infected clones (BUB3-knockdown-HeLa and SEPT10-knockdown-HeLa), a negative control vector (pFIV-H1)-transfected HeLa clone (vehicle), and non-transfected HeLa clone (wild type) with 10-nM paclitaxel, a concentration that corresponded to the paclitaxel-resistant colonies, and examined the number of surviving cells. As expected, the knockdown clones delayed the doubling time relative to their parental cells; nevertheless, these cells survived better than the controls (Fig. 1a,b). We also confirmed by Western blot analysis decreases in BUB3 and SEPT10

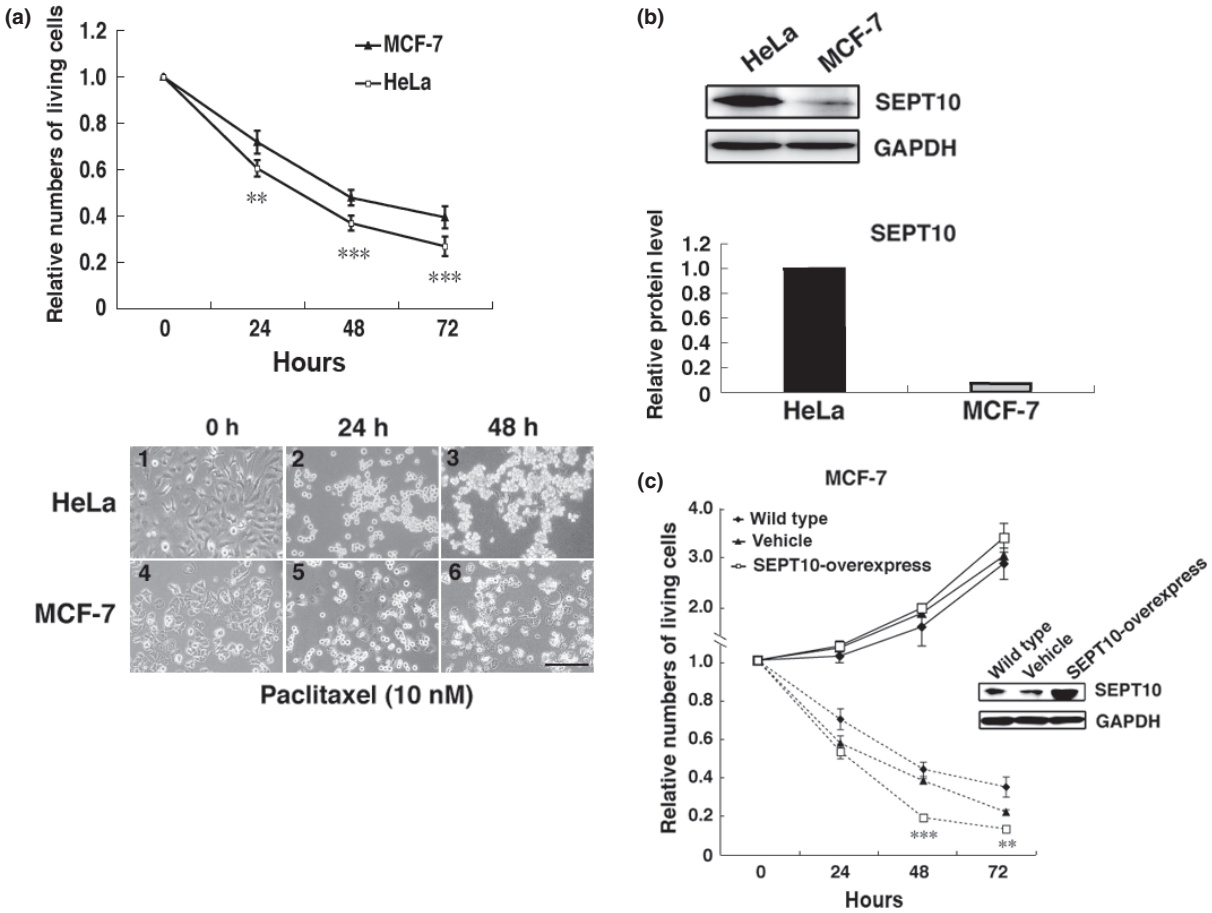

Fig. 2. Expression level of SEPT10 is important for paclitaxel sensitivity. (a) MCF-7 cells were more resistant to paclitaxel compared with HeLa cells. HeLa and MCF-7 cells were cultured, and the living cell numbers were counted at 0, 24, 48 and 72 h after the addition of paclitaxel. Microscopic images of HeLa and MCF-7 cells at 0, 24 and 48 h are also displayed. Scale bar, 100 μm. (b) The SEPT10 expression level in MCF-7 is much lower than in HeLa cells. Total protein samples from HeLa and MCF-7 cells were subjected to Western blotting. A graphical representation of SEPT10 protein levels is also shown. (c) The SEPT10 expression vector-transfected MCF-7 stable clone (SEPT10-overexpress) showed increased sensitivity to paclitaxel compared to the wild-type and control vector-transfected (vehicle) clones. The cell proliferation of SEPT10-overexpress MCF-7 showed no significant differences compared to its vehicle and wild type at 0, 24, 48 and 72 h. The solid lines indicate paclitaxel treated, and the dotted lines are non-treated. SEPT10 expression levels in the clones were also confirmed by Western blotting. $^{**}P < 0.01$; $^{***}P < 0.001$. MCF-7, Michigan Cancer Foundation-7; SEPT10, septin 10.

protein levels in the knockdown clones compared to the control cells (Fig. 1a). Furthermore, we examined whether the chemically synthesized siRNA specific to *BUB3* and *SEPT10* also reduced paclitaxel sensitivity. In this experiment, the siRNA target sites in the *BUB3* and *SEPT10* mRNA were different from the shRNA-targeted sites in BUB3-knockdown-HeLa and SEPT10-knockdown-HeLa clones. We confirmed that the active siRNA made HeLa cells paclitaxel resistant (Fig. 1c). These data demonstrate that suppressing BUB3 or SEPT10 produces paclitaxel resistance.

Overexpression of SEPT10 increased paclitaxel sensitivity. Paclitaxel is often clinically used to treat cancers, such as breast and ovarian, so we used MCF-7 for a breast cancer and HeLa cells for a cervical cancer. Interestingly, we found that MCF-7 was less sensitive to paclitaxel and had a lower SEPT10 expression level than HeLa cells (Fig. 2a,b). These results prompted us to examine whether increased SEPT10 expression makes MCF-7 more sensitive to paclitaxel. We treated SEPT10-expression vector (SEPT10-pDEST40)-transfected MCF-7 clone (SEPT10-overexpress), pDEST40 vector-transfected MCF-7 clone (vehicle) and non-transfected MCF-7 clone (wild type) with 10-nM paclitaxel. As expected, there were fewer viable SEPT10-overexpress cells, and the number decreased more rapidly and substantially than control cells. However, cells without paclitaxel treatment increased more in the SEPT10-overexpress cells than in the vehicle or wild-type cells (Fig. 2c). These results show that the expression level of SEPT10 is significant for paclitaxel sensitivity.

Low-level expression of SEPT10 increases microtubule stability. Paclitaxel induces an accumulation of cells at G2/M and a sub-G1 apoptotic region.[22] We examined whether SEPT10 expression levels affect the cell cycle profile after paclitaxel treatment. We treated SEPT10-knockdown and overexpressed clones and their negative control clones of HeLa and MCF-7 with 10-nM paclitaxel for 24 h and performed flow cytometric analysis. Although control HeLa cells (vehicle) were arrested at the G2/M phase by paclitaxel treatment, SEPT10-knockdown-HeLa cells were arrested at various cell cycle phases (Fig. 3a). In contrast, control MCF-7 cells (vehicle) with low level SEPT10 expression was not arrested at G2/M phase by the paclitaxel treatment, but SEPT10-overexpressed-MCF-7 cells showed an increase in arrested cells at G2/M phase (Fig. 3b). These data suggest that SEPT10 expression level may be important for spindle checkpoint.

In addition, it was reported that expression of SEPT7 destabilizes microtubules *in vivo*.[15] Thus, we investigated whether loss of SEPT10 also affects microtubule stability. To examine this stabilizing effect, non-transfected (wild-type), control vector-transfected (vehicle) and SEPT10-knockdowned HeLa cells were treated with 10-nM nocodazole, a microtubule-depolymerizing reagent, for 30 min and then stained with anti-β-tubulin antibody. As seen in Figure 3c, unlike SEPT10-knockdown cells after nocodazole treatment, vehicle and wild-type cells did not maintain their distribution in the microtubule array and began to cluster. However, in the MCF-7 cell line that expressed low levels of SEPT10, the clone carrying a SEPT10-expression vector (SEPT10-overexpress) showed damaged microtubule stability, but wild-type MCF-7 cells and the cells carrying a control vector (vehicle) did not show significant damage after nocodazole treatment (Fig. 3d). These data indicated that SEPT10 regulates microtubule stabilities as well as other members of the septin family.

Association between SEPT10 and MAP4. Mammalian septins reduce microtubule stability through interaction with

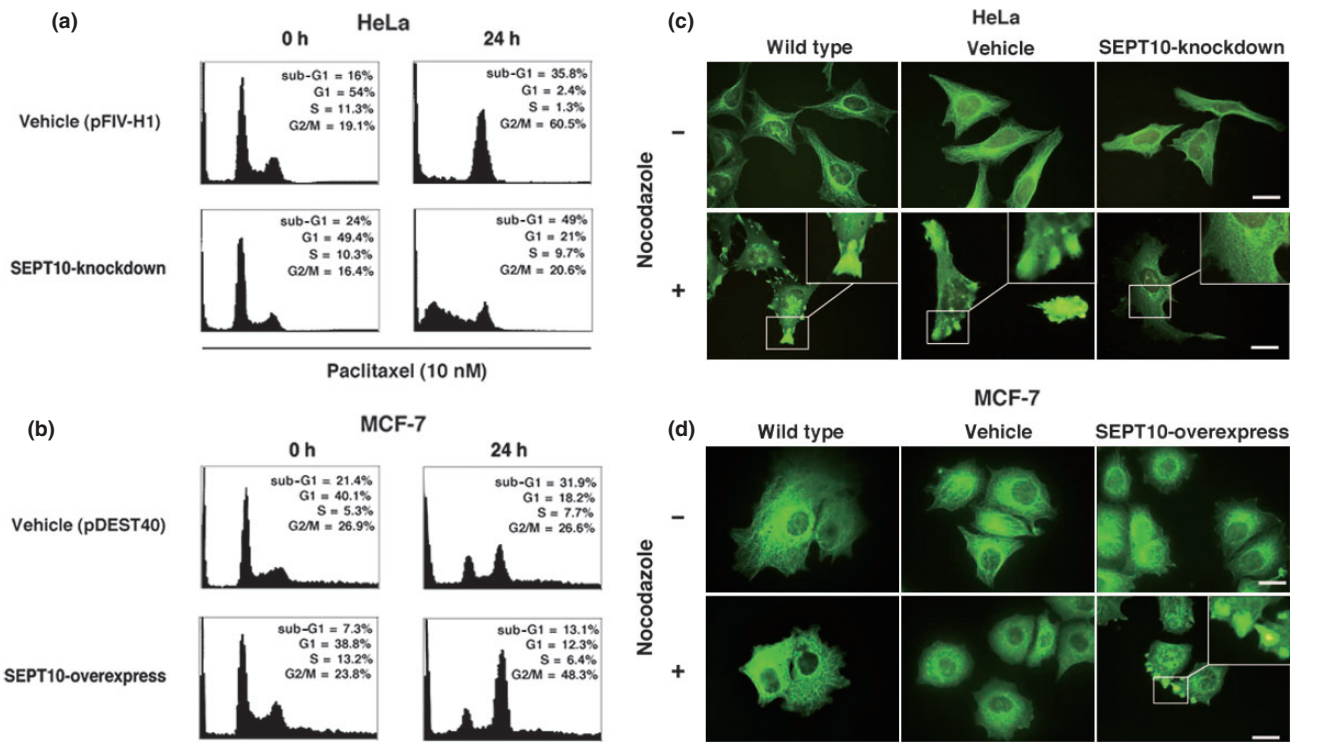

Fig. 3. SEPT10 reduces microtubule stability. Flow cytometric analysis of SEPT10-knockdown and negative control (vehicle) clones of HeLa (a) and SEPT10 overexpression (SEPT10-overexpress) and negative control (vehicle) clones of MCF-7 (b). The cells were treated with 10-nM paclitaxel, and then harvested at 0 and 24 h after addition of paclitaxel. SEPT10-knockdown-HeLa cells increased microtubule stability (c) and SEPT10-overexpressed MCF-7 cells (SEPT10-overexpress) increased nocodazole sensitivity (d) during nocodazole treatment. Cells were cultured with 10-nM nocodazole for 30 min, and were stained with an anti-β-tubulin antibody. Scale bar, 10 μm. MCF-7, Michigan Cancer Foundation-7; S, synthesis phase of cell cycle; SEPT10, septin 10.

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MAP4.[15] Thus, we examined MAP4 expression in SEPT10-knockdowned HeLa cells after paclitaxel treatment. We observed increased expression of MAP4 in control cells (vehicle) but did not in the SEPT10-knockdowned cells (Fig. 4a). We further examined by IP analysis with an anti-MAP4 antibody whether SEPT10 binds to MAP4 in the HeLa clones of both SEPT10-knockdown and vehicle after paclitaxel treatment. Interestingly, we observed the interaction between SEPT10 and MAP4 in SEPT10-knockdown-HeLa cells but not in control (vehicle) HeLa cells (Fig. 4b). Additionally, MCF-7 cells that expressed low levels of SEPT10 were associated twith MAP4 (Fig. 4c). These data showed that SEPT10 binds to MAP4 but only when SEPT10 is expressed at low level.

Decreased expression of SEPT10 and caspase-3 in paclitaxel-resistant ovarian carcinoma specimens. Finally, to examine whether SEPT10 expression was associated with clinical paclitaxel resistance, we performed immunohistochemical analysis on clinical specimens of ovarian carcinoma. It has reported that paclitaxel-sensitive cancers show high-level expression of caspase-3.[23] Thus, we also examined expression of caspase-3, a downstream effector of apoptosis, as an index of the specimens' paclitaxel sensitivity. As shown in Figure 5, SEPT10 and caspase-3 were strongly expressed in cytoplasm of paclitaxel-sensitive tumor cells. In contrast, carcinoma specimens from paclitaxel-resistant patients showed significantly lower expression of SEPT10 ($P < 0.05$) and caspase-3 ($P < 0.05$).

Discussion

Paclitaxel is one of the most successful drugs for the treatment of cancer,[24] but many patients show resistance to paclitaxel. Previous studies have identified several different mechanisms involved in paclitaxel resistance,[2–7,25,26] and these results are expected to contribute the development of future cancer treatments. In this study, using a lentiviral siRNA library, we isolated two genes, *BUB3* and *SEPT10*, that encode molecules involved in a mechanism of paclitaxel sensitivity (Fig. 1). It has been already reported that siRNA knockdown of BUB3 blocks paclitaxel-induced cell cycle arrest,[21] suggesting that our strategy works well for identifying genes involved with paclitaxel sensitivity. However, SEPT10 is novel for paclitaxel resistance. We have also revealed that SEPT10 is involved in microtubule stability (Fig. 3). Because the primary target of paclitaxel is microtubules, it is natural that molecules related to paclitaxel resistance are involved in microtubule-related mechanisms. It is possible that other molecules functioning in microtubule-related mechanisms will also be identified as novel paclitaxel-sensitive molecules.

Our results have shown that the expression level of SEPT10 affects paclitaxel sensitivity (Figs 1,2,5). Interestingly, all cases of paclitaxel-resistant ovarian carcinomas examined in this study show decreased expression of SEPT10 (Fig. 5). However, there are several mechanisms involving different molecules for paclitaxel resistance.[2–7,21,25,26] Therefore, these results suggest two possibilities: (i) at least in ovarian cancer, paclitaxel resistance in many cases is caused by SEPT10 depletion, and (ii) SEPT10 may play a critical role in paclitaxel resistance or may cooperate with other molecules in various paclitaxel-resistant mechanisms. Regardless, we expect the expression level of SEPT10 will become a promising clinical marker for paclitaxel resistance. In contrast, overexpression of SEPT10 makes cells more sensitive to paclitaxel (Fig. 2c), indicating that induction of SEPT10 expression may be useful for treating cancer in paclitaxel-resistant patients. The discovery promising chemicals or the development of novel gene therapy methods to activate SEPT10 expression may be significant to the future of chemotherapy.

We observed that MAP4 induction by paclitaxel treatment requires high-level expression of SEPT10 (Fig. 4a). However,

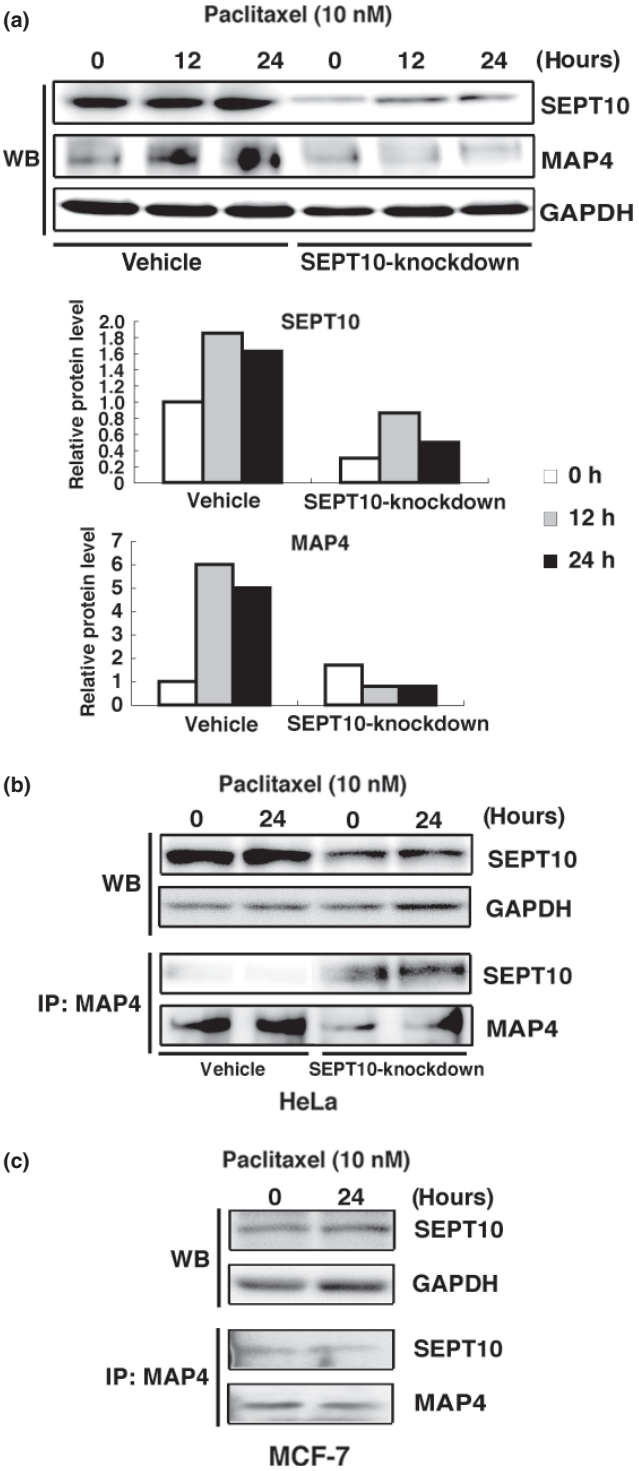

Fig. 4. Association between SEPT10 and MAP4. (a) Knockdown of SEPT10 inhibited MAP4 induction by paclitaxel. SEPT10 siRNA-transfected HeLa cells and control siRNA-transfected HeLa cells were treated with 10-nM paclitaxel and harvested at 0, 12 and 24 h after addition of paclitaxel. Then, total protein samples from the cells were subjected to Western blotting. A graphical representation of SEPT10 and MAP4 protein levels is also shown. (b) Only in the SEPT10-knockdown HeLa cells was SEPT10 associated with MAP4. The HeLa clones of SEPT10-knockdown and vehicle were treated with 10-nM paclitaxel and then harvested at 0 and 24 h after addition of paclitaxel. The harvested samples were subjected to Western blotting and immunoprecipitation assay. (c) Wild-type MCF-7 cells, which express low levels of SEPT10, bound to MAP4. IP, immunoprecipitation; MAP4, microtubule-associated protein 4; MCF-7, Michigan Cancer Foundation-7; SEPT10, septin 10; WB, Western blotting.

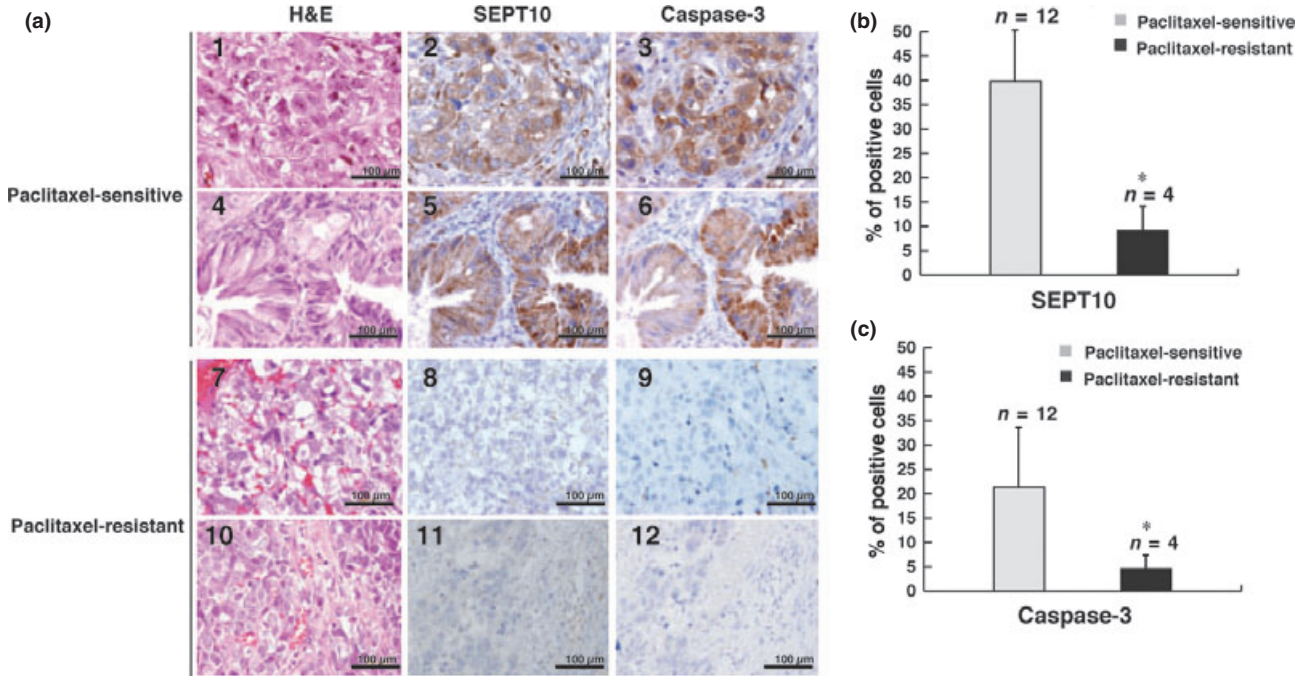

Fig. 5. Decreased expression of SEPT10 and caspase-3 in paclitaxel-resistant ovarian carcinoma specimens. (a) Immunohistochemistries of paclitaxel-sensitive and paclitaxel-resistant cases with anti-SEPT10 and anti-caspase-3 antibodies. SEPT10 is shown in brown in the panels 2, 5, 8 and 11, and caspase-3 is shown in brown in the panels 3, 6, 9 and 12. Scale bars, 100 μm. (b,c) The frequencies of immunostainpositive cells per 500 tumor cells is quantified. $*P < 0.05$. SEPT10, septin 10.

IP analysis showed that SEPT10 bound to MAP4 only when the expression level of SEPT10 was low (Fig. 4b). At present, we do not know which mechanism explains these results. MAP4 is a multifunctional protein that plays important roles in the overall regulation of microtubule cytoskeleton. Kremer *et al.*[15] showed that mammalian septins, including SEPT2, SEPT6, and SEPT7, bind to MAP4 and inhibit the binding activity of MAP4 to microtubules, reducing the stability of cellular microtubules. In contrast, an essential feature of microtubule activity is dynamic instability. Paclitaxel binds to the β-tubulin subunit in polymerized microtubules, resulting in the suppression of microtubule dynamics and the stabilization of the microtubules themselves.[27] In short, the depletion of septins has the potential to cancel the effects of paclitaxel on microtubules. Thus, the results of the IP analysis are consistent with the results that cells exhibit paclitaxel resistance only when SEPT10 is expressed at low levels (Fig. 4b). The mechanism of binding between SEPT10 and MAP4 remains unclear and requires further elucidation in the near future.

In summary, we have revealed that SEPT10 expression level is important for spindle checkpoints and paclitaxel sensitivity. SEPT10 will probably become a good biomarker for paclitaxel resistance, and its related mechanism may be useful in novel therapeutic methods including gene therapy and chemotherapy for cancer. We strongly expect that our results will contribute future cancer therapy.

Acknowledgments

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Disclosure Statement

The authors declare no conflict of interest.

Abbreviations

BUB3	budding uninhibited by benzimidazoles 3
FIV	feline immunodeficiency virus
IP	immunoprecipitation
MAP4	microtubule-associated protein 4
SEPT	septin
USP15	ubiquitin-specific protease 15

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