Functional role of tau protein in epithelial ovarian cancer cells
（上皮性卵巣癌細胞におけるタウ蛋白の機能）

申請者　　弘前大学大学院医学研究科
腫瘍制御科学領域　婦人科腫瘍学教育研究分野

氏　名　　山内　愛紗

指導教授　　横山　良仁

ABSTRACT

Purpose Microtubule-associated protein tau (MAPT) is a marker of paclitaxel sensitivity in ovarian cancer (OC). The aim of the present study was to elucidate the function of tau protein in epithelial OC.

Methods We investigated the correlation between tau protein expression and response to paclitaxel using several OC cell lines.

Results Western blot showed that the expression level of tau protein was the highest in TOV112D cells. We compared the proliferation rates of control cells to those with downregulated tau by using cell counting Kit-8 and found that cell proliferation was more inhibited in the cells with downregulated tau than in control cells, both with and without paclitaxel treatment. We also compared the proliferation rates of control cells and TOV112D cells with tau protein overexpression. Cell proliferation was more inhibited in cells overexpressing tau compared to control cells, both with and without paclitaxel treatment. We showed that both the downregulation and the overexpression of tau protein were related to the inhibition of TOV112D cell proliferation. Early and late apoptosis in TOV112D transfected with tau cDNA plasmid construct or tau siRNA significantly increased.

Conclusions These findings suggest that molecular targeting of the tau protein could be a potential treatment for OC.

Key words: Epithelial ovarian cancer, Tau protein, paclitaxel, cell proliferation, apoptosis.

Introduction

Ovarian cancer (OC) is the leading cause of death among gynecological cancer patients. Paclitaxel and platinum-based chemotherapy is often used as first-line chemotherapy in the treatment of epithelial OC patients. Almost all epithelial OC patients are good responders to paclitaxel and platinum-based chemotherapy. However, some patients experience relapse and require second-line chemotherapy. A useful marker of sensitivity to paclitaxel and platinum-based chemotherapy had not been previously identified.

It is well known that the tau protein is related to Alzheimer disease. Accumulation of hyperphosphorylated and aggregated microtubule-associated protein tau (MAPT) is a central feature of a class of neurodegenerative diseases termed tauopathies. The continuing increase in the adult-born neurons indicates that tauopathies are characterized by increased neurogenesis [1]. The molecular weight of the tau protein is 50-64 kDa, and it is a product of the *TAU* gene located in chromosome 17. In addition to neurons, tau is expressed at low levels in several non-neuronal cells. The tau protein consists of an N-terminus and a C-terminus. It has a microtubule binding part at the C-terminus. In the mammalian brain, the tau protein has 6 isoforms that differ in the number of microtubule-binding domain repeats (3 or 4) [2]. The tau protein binds to beta-tubulin and thus stabilizes the microtubules. Paclitaxel exhibits its effect through the exact same mechanism. Therefore, the tau protein and paclitaxel compete for binding to the microtubules.

Recently, the tau protein has been identified as a marker of response to paclitaxel in breast cancer and OC [3, 4]. Smoter et al. reported that negative expression of tau protein appears to be both a good prognostic factor and a predictor of response to paclitaxel and platinum-based chemotherapy in epithelial OC patients [4]. Rouzier et al. also reported that low tau protein expression might be used as a marker to select patients for paclitaxel therapy [5]. In the field of gynecological oncology, very little work has been

done on the tau protein. As described above, the tau protein and paclitaxel bind to the same part of microtubules, thereby promoting tubulin polymerization and microtubule stabilization. This is the mechanism of the antitumor action of paclitaxel. In the field of neurology, it is well known that phosphorylation of tau protein causes apoptosis of neurons; the tau protein is closely related with cell apoptosis. Therefore, we researched the function of the tau protein with respect to the effect of paclitaxel. We selected the OC cell line because paclitaxel and platinum-based chemotherapy is the first-line chemotherapy regimen for epithelial OC. However, the role of tau protein in cancer cells has been clarified yet. Therefore, we analyzed tau protein function in epithelial OC cells in this study.

Materials and methods

Cell lines and cell culture

TOV112D, MCAS, and OVCAR3 cell lines were obtained from the American Type Culture Collection (Rockville, MD). TOV112D is derived from human endometrioid carcinoma [6], MCAS is derived from human mucinous carcinoma [7], and OVCAR-3 is derived from human serous carcinoma [8]. OVICE cell line derived from human clear cell carcinoma was obtained from Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan) [9]. HRA cells and DISS cells, derived from human epithelial ovarian carcinoma, were generously provided by Dr. Y. Kikuchi (National Defense Medical College, Japan) [10] and Dr. Saga (Jichi Medical School, Tochigi, Japan) [11], respectively. All cells were grown in Roswell Park Memorial Institute (RPMI) medium-1640 Medium (Sigma-Aldrich, St Louis, MO) supplemented with 10% fetal bovine serum, at 37°C, in a water-saturated atmosphere with 5% CO_2/95% air.[12] All cell lines were verified in writing as being ovarian in origin and no mycoplasma contamination [12].

Plasmid DNA preparation

We used a pCMV6-AC-GFP vector (OriGene Technologies, Inc., Rockville, MD) that encodes the human

MAPT transcript variant 1 fused to green fluorescent protein (GFP) and ampicillin resistance gene. For amplification, pCMV6-AC-GFP was transformed into DH5α competent cells via heat-shock transformation, according to the standard laboratory protocols [13]. The transformed bacteria were amplified in LB-ampicillin medium [13]. The plasmids were purified from cultured transformed bacteria using a PureLink HiPure Plasmid Filter Maxiprep DNA purification kit (Invitrogen Life Technologies, Carlsbad, CA), according to the manufacturer's instructions [13]. Plasmid DNA was diluted in sterile water at a concentration of 3 μg/μl [13].

Small interfering RNA preparation

The sequence of the small interfering RNA (siRNA) duplex specific for MAPT was synthesized commercially by OriGene Technologies. We briefly centrifuged the siRNA tube to ensure that all contents collected at the bottom of the tube, and we re-suspended the duplexes in the provided RNase-free duplex buffer. The tube was heated to 94°C for 2 min and subsequently cooled to room temperature.

In vitro plasmid DNA transfection

One day before transfection, 0.5×10^7 TOV112D and HRA cells were plated and cultured in 15 ml of RPMI medium-1640 Medium supplemented with 10% fetal bovine serum without antibiotics in 10-cm culture dishes, so that they would reach 80% confluence at transfection [12]. We used Lipofectamine 3000 (Thermo Fisher Scientific, Waltham, MA) for DNA transfection. We diluted 24 μg of MAPT DNA in 1000 μl phosphate-buffered saline (PBS) and 60 μl of lipofectamine in 1000 μl PBS. We then incubated the dilutions for 5 min at room temperature. After the incubation, we combined the diluted DNA lipofectamine and incubated for 20 min at room temperature. After the incubation, we added 2000 μl of complexes to each 10-cm culture dish. The vector without MAPT DNA was used as control. The control cells were only given mixture of the vector and lipofectamine.

In vitro siRNA transfection

One day before transfection, 0.2×10^7 TOV112D and OVCAR3 cells were plated and cultured in 15 ml of

Roswell Park Memorial Institute medium-1640 Medium supplemented with 10% fetal bovine serum without antibiotics in 10-cm culture dishes, so that they would reach 50% confluence at transfection [12]. We used Lipofectamine 3000 for siRNA transfection. First, we diluted 600 pmol MAPT siRNA in 1000 µl of PBS and 30 µl of lipofectamine in 1000 µl PBS. We then incubated the dilutions for 5 min at room temperature. Next, we combined the diluted siRNA with diluted lipofectamine and incubated for 20 min at room temperature. After the incubation, we added 2000 µl of complexes to each 10-cm culture dish. Control siRNA-A (sc-36869, Santa Cruz Biotechnology, Santa Cruz, CA) instead of MAPT siRNA served as a control.

Western blot analysis

Cell lysates were prepared from TOV112D, OVCAR3, HRA, DISS, OVISE and MCAS cells. In addition, cell lysates were prepared from TOV112D and HRA cells cultured with a solution of lipofectamine/MAPT DNA complex for 48 h and TOV112D and OVCAR3 cells cultured with a solution of lipofectamine/MAPT siRNA complex for 48 h. The protein concentration was measured using the Bradford method [14]. The protein samples (50 μg) were run through 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis [14]. After the electrophoretic transfer of the protein to nitrocellulose membrane, nonspecific binding was blocked by incubation with 5% skim milk in 20 mm Tris-HCl, pH 7.5, 0.5 M NaCl (TBS) for 1 h at room temperature [14]. After being washed three times with TBS containing 0.05% Tween 20 (TTBS), the blots were probed with a 1:200 dilution of rabbit polyclonal IgG specific for human MAPT (sc-5587, Santa Cruz Biotechnology) for 2 h [14]. The blots were also probed with a 1:1000 dilution of monoclonal anti-β-actin antibody (Sigma-Aldrich) to be relatively quantified. β-Actin was used as a loading control [14]. The membranes were then washed three times with TTBS and incubated for 1 h at room temperature with an anti-rabbit IgG HRP-linked antibody (Cell Signaling Technology, Beverly, MA) for MAPT and an anti-mouse immunoglobulin G HRP-linked Antibody (Cell Signaling Technology) for β-actin, respectively [14]. After being washed three times with TTBS, the

membrane was transferred to ECL Western blotting Detection Reagents (GE Healthcare, Buckinghamshire, UK) and incubated in this solution for 60 s at room temperature [14]. The protein bands on the membrane were visualized using enhanced chemiluminescence (ChemiDoc XRS, Biorad, Hercules, CA) according to the manufacturer's instructions [12]. The band intensity was analyzed with Molecular Imager, Image Lab Ver. 3.0.1 (Bio-Rad, Hercules, CA).

Cell proliferation assay

Cell proliferation was assayed using a cell counting Kit-8 (CCK-8; Dojin Laboratories, Kumamoto, Japan) [12]. TOV112D and HRA cells were cultured overnight in 96-well microplates at 4.0×10^4 cells per well with 100 μl of medium. Afterwards, a solution of lipofectamine/DNA complex containing 0.1 μg of human MAPT DNA was added to the cells. The control group was given only mixture of the vector and lipofectamine. Transfected cells were incubated at 37°C in a CO_2 incubator for 48 h [12]. Afterwards, paclitaxel was added to the cells in 4 pattern concentrations—0, 1, 10, and 50 μg/ml—and incubated at 37°C in a CO_2 incubator for 48 h. Cell proliferation was assessed 4 h after the addition of CCK-8 by measuring A_{450} with a Multiskan FC microplate reader (Thermo scientific, Yokohama, Japan) [12]. A preliminary study using this kit showed that absorbance was directly proportional to the number of cells [12]. The experiment was conducted in triplicate. The proliferation of TOV112D and OVCAR3 cells also assessed adding a solution of lipofectamine/siRNA complex containing 5 pmol human MAPT siRNA.

Cell cycle analysis

Cell cycle analysis was performed using the Cell Cycle Phase Determination Kit (Cayman chemical, MI). The TOV112D (control), MAPT DNA-transfected TOV112D, and MAPT siRNA-transfected TOV112D cells were collected and centrifuged to pellet them at the bottom. After centrifugation, we washed the cells twice with assay buffer and resuspended the cell pellet to a density of 10^6 cells/mL in Assay Buffer. In order to fix and permeabilize the cells, we added 1 mL of fixative to each sample. Next, we centrifuged the fixed cells at 500 × g for 5 min, and decanted the fixative. Suspended the cell pellet in 0.5 mL of

staining solution and incubated for 30 min at room temperature. The cell cycle was analyzed in the FL2 channel of a flow cytometer with a 450-nm excitation laser.

Apoptosis analysis

Apoptosis analysis was performed using the Annexin V-FITC Kit System for Detection of Apoptosis (Beckman Coulter, CA). The TOV112D (control), MAPT DNA-transfected TOV112D, and MAPT siRNA-transfected TOV112D cells were collected, and washed with PBS after centrifugation for 5 min at 500 × g and 4°C. We discarded the supernatant and resuspended the cell pellet in diluted binding buffer to 5×10^5 cells/mL. Next, we added 5 µl of Annexin V-FITC solution and 2.5 µl dissolved propidium iodide to 100 µl of the cell suspension. We kept the tube on ice and incubated the tubes for 10 min in the dark. We added 400 µl of binding buffer and analyzed the cell samples by flow cytometry.

Statistical analysis

All statistical analyses were performed using the Student t-test, and all P-values < 0.05 were considered statistically significant.

Results

Expression levels of tau protein in various cell lines

Western blot analysis showed that the expression level of tau protein was the highest in TOV112D cells (Fig. 1), whereas it was expressed weakly in OVCAR3 cells. On the other hand, it was residually expressed in other 4 cell lines. MAPT includes 6 isoforms between 46-80 kDa and its antibody detects multiple bands in the range. Fig. 2A shows a comparison of the tau protein expression in TOV112D control cells and MAPT DNA-transfected TOV112D cells and in HRA control cells and MAPT DNA-transfected HRA cells. We performed a similar comparison between TOV112D control and siRNA-transfected TOV112D cells and OVCAR3 control and MAPT siRNA- transfected OVCAR3 cells (Fig. 2B). In the TOV112D and HRA cells, transfection with MAPT DNA increased the expression of tau

protein whereas in TOV112D and OVCAR3 cells, transfection with MAPT siRNA decreased the expression of tau protein (Fig. 2A and B). In particular, transfection of MAPT DNA showed multiple bands and its phenomenon suggested the hyper-phosphorylation [15].

Changes in cell proliferation after MAPT DNA or MAPT siRNA transfection

Fig. 3 shows the changes in cell proliferation, assessed by measuring A_{450} with a Multiskan FC microplate reader 4 h after the addition of CCK8. The proliferation of MAPT DNA-transfected TOV112D cells was significantly more inhibited than that of the control cells at paclitaxel concentrations of 0, 1, and 10 μg/ml (Fig. 3A). The proliferation of MAPT DNA-transfected HRA cells was not inhibited compared to that of the control cells at almost all paclitaxel concentrations (Fig. 3B). The proliferation of MAPT siRNA- transfected TOV112D cells was significantly more inhibited than that of the control cells at paclitaxel concentrations of 0, 0.1, 1, and 10 μg/ml (Fig. 4A). The proliferation of MAPT siRNA-transfected OVCAR3 cells was not inhibited compared to that of the control cells at almost all paclitaxel concentrations (Fig. 4B). Note here that MAPT DNA-transfection or MAPT siRNA-transfection significantly inhibited cell proliferation of TOV112D without administration of paclitaxel.

Cell cycle changes after MAPT DNA or MAPT siRNA transfection

Because MAPT DNA-transfection or MAPT siRNA- transfection altered cell proliferation of TOV112D, we analyzed the cell cycle in TOV112D cells with their transfection. The average apoptosis rate in control cells was 4.1%, and the average apoptosis rates of cells transfected with DNA and siRNA were 29.0% and 11.7%, respectively. The rate of apoptosis increased significantly in cells transfected with MAPT DNA or siRNA ($p < 0.05$, Fig. 5).

Alteration of Apoptosis after MAPT DNA or MAPT siRNA transfection

While Annexin-V+/PI- cells show early apoptosis, Annexin-V+/PI+ cells show late apoptosis. In the control cells, the average rate of total apoptosis was 12.8% (average early apoptosis, 11.3%; late apoptosis, 1.5%). In DNA-transfected cells, the average total was 72.8% (average early apoptosis rate,

52.6%; average late apoptosis, 19.7%). In siRNA-transfected cells, the average rate of total apoptosis was 29.9% (average early apoptosis, 27.6%; average late apoptosis, 2.3%). The rates of apoptosis increased significantly in DNA-transfected cells and in siRNA-transfected cells ($p < 0.0001$ and $p < 0.001$, Fig. 6).

Discussion

First, we researched the level of tau protein expression in various OC cells. As shown in Fig. 1, the tau protein expression level is different according to the cell type. The level of tau expression was the highest in TOV112D cells. TOV112D cells are a type of endometrioid carcinoma. Therefore, tau protein expression levels may be higher in endometrioid carcinoma than in other types of OC. OVCAR3, DISS, and HRA are types of serous carcinoma. OVISE is a type of clear cell carcinoma. MCAS is a type of mucinous carcinoma. In a previous study, Smoter et al immunohistochemically stained primary tumors of 74 OC patients for the tau protein [4]. They compared the number of tau-positive patients between patients with the serous and those with the nonserous type. However, they reported that there is no significant difference between the histological types. In our study, there was a difference in tau expression levels among patients with the same histological type. HRA and OVCAR3 cells are types of serous carcinoma, and there are many other histological types of OC cells. Accordingly, we need to research tau protein level expression in other cell types. Gurler et al. compared the level of tau protein expression among 5 types of serous OC cells [16]. The level of tau protein expression was different in each type [16]. The reason for the difference of tau protein expression among the histological types or among individuals is unclear [16]. Caillet-Boudin et al. reported that repeated sequences, CpG islands, and haplotypes affect DNA and RNA levels of MAPT, and regulate MAPT gene expression [17]. These factors may have different actions among cells of each histological type.

The tau protein has recently been identified as a marker of response to paclitaxel in breast cancer [5, 18-22]. The tau protein and paclitaxel compete for binding to microtubules. Therefore, the sensitivity to

paclitaxel should be weak in cells with high tau protein expression. Smoter et al examined the tau expression of 74 epithelial OC patients, and compared the progression-free survival (PFS) and 3-year overall survival (OS) [4]. The PFS of tau-negative patients was significantly better than that of tau-positive patients (p=0.0355). In univariate analysis, the 3-year OS of tau-negative patients was significantly better than that of tau-positive patients (p=0.0198) [4]. However, in multivariate analysis, there was no significant difference [4]. They concluded that negative tau protein expression seems to be both a good prognostic factor and a predictor of response to paclitaxel and platinum-based chemotherapy in epithelial OC patients [4]. Conversely, Steffensen et al. reported that tau expression was not associated with PFS or OS in serous epithelial OC [23]. Furthermore, many other factors affect PFS and OS, including the histological type, stage, residual tumor size, and age. Chemosensitivity is also influenced by many factors [24-28]. In epithelial OC patients, sensitivity to both paclitaxel and platinum affect response to chemotherapy. Therefore, we investigated the relationship between paclitaxel use and tau protein expression in epithelial OC cells. We expected that the regulation of cell proliferation by paclitaxel would become stronger by transfection of MAPT siRNA (tau downregulation). We also expected that the regulation of cell proliferation by paclitaxel would become weaker by transfection of MAPT DNA. As shown in Fig. 4, in TOV112D cells, cell proliferation was more inhibited by transfection of MAPT siRNA. We specifically noted that cell proliferation was significantly inhibited in cells with MAPT siRNA transfection without paclitaxel. The transfection of MAPT siRNA resulted in regulation of cell proliferation. In OVCAR3 cells, similar changes were not observed because the level of tau protein expression in OVCAR3 cells was originally low. Among TOV112D cells, the cell proliferation of MAPT DNA transfected cells was also more inhibited without paclitaxel (Fig. 3). Thus, downregulation and overexpression of tau protein regulate cell proliferation. In HRA cells with low levels of tau protein expression, similar changes were not observed. We made an anticipation that downregulation of the tau protein would cause inhibition of polymerization of microtubules. We also made an anticipation that

overexpression of tau protein would cause inhibition of de-polymerization of microtubules. Both these processes lead to apoptosis of cells. Fig. 6 showed the increase in cell apoptosis of TOV112D cells transfected with MAPT DNA and siRNA. Apoptosis increased in both MAPT DNA-transfected cells and MAPT siRNA-transfected cells, but the ratio of late apoptosis to total apoptosis increased in MAPT DNA-transfected cells. This may be because the mechanism of cell apoptosis is different between MAPT DNA-transfected cells and MAPT siRNA-transfected cells. We speculate that the cytostatic effect by transfection of MAPT DNA is more rapid and stronger compared with that by transfection of MAPT siRNA. The hyper-phosphorylation of MAPT may deprive its binding ability to microtubules and cause apoptosis [1]. In fact, MAPT DNA transfection led to the hyper-phosphorylation of MAPT as shown in Fig. 2A. However, the relevance of the increase in the ratio of late apoptosis is unclear.

Inhibition of cell proliferation by the transfection of MAPT DNA or siRNA was observed only in TOV112D cells. The reason for this inhibition may be the high level of tau protein expression in TOV112D cells. The extent of contribution of the tau protein in cell proliferation may differ in each cell line. The limitation of this study is that such a phenomenon was demonstrated just in TOV112D cells. In order to indicate a possibility of new molecularly targeted therapy in tau-positive cells, further studies should confirm the cytostatic effect by the downregulation and overexpression of tau proteins by using another cell lines such as breast cancer and neurological malignancy with high tau protein expression. The details of the apoptosis mechanism also need to be elucidated. An obvious limitation of this study is that there is no examination of clinical samples. Because the phenotype of the cell lines does not always reflect their original characters, future study should show a relationship between apoptosis and MAPT distribution and expression in the clinical samples of OC. If this is done, the cytostatic effect by the downregulation and overexpression of tau protein can be analyzed in vivo.

Acknowledgements

This study was supported by a Grant-in-Aid for Cancer Research from the Ministry of Education, Culture, Sports, Science and Technology (Tokyo, Japan) (no. 20591935 to Dr Y. Yokoyama).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Ethical consideration

Human /Animal rights: This article does not contain any studies with human and animal subjects performed by the any of the authors.

Approval by Ethics Committee: Authors must state in the Disclosures section that the protocol for the research project including human subjects has been approved by a suitably constituted Ethics Committee.

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Legends for Figures

Figure 1. Western blot analysis for tau protein expression levels. The level of tau protein expression was the strongest in TOV112D cells. MAPT includes 6 isoforms between 46-80 kDa and its antibody detects multiple bands in the range.

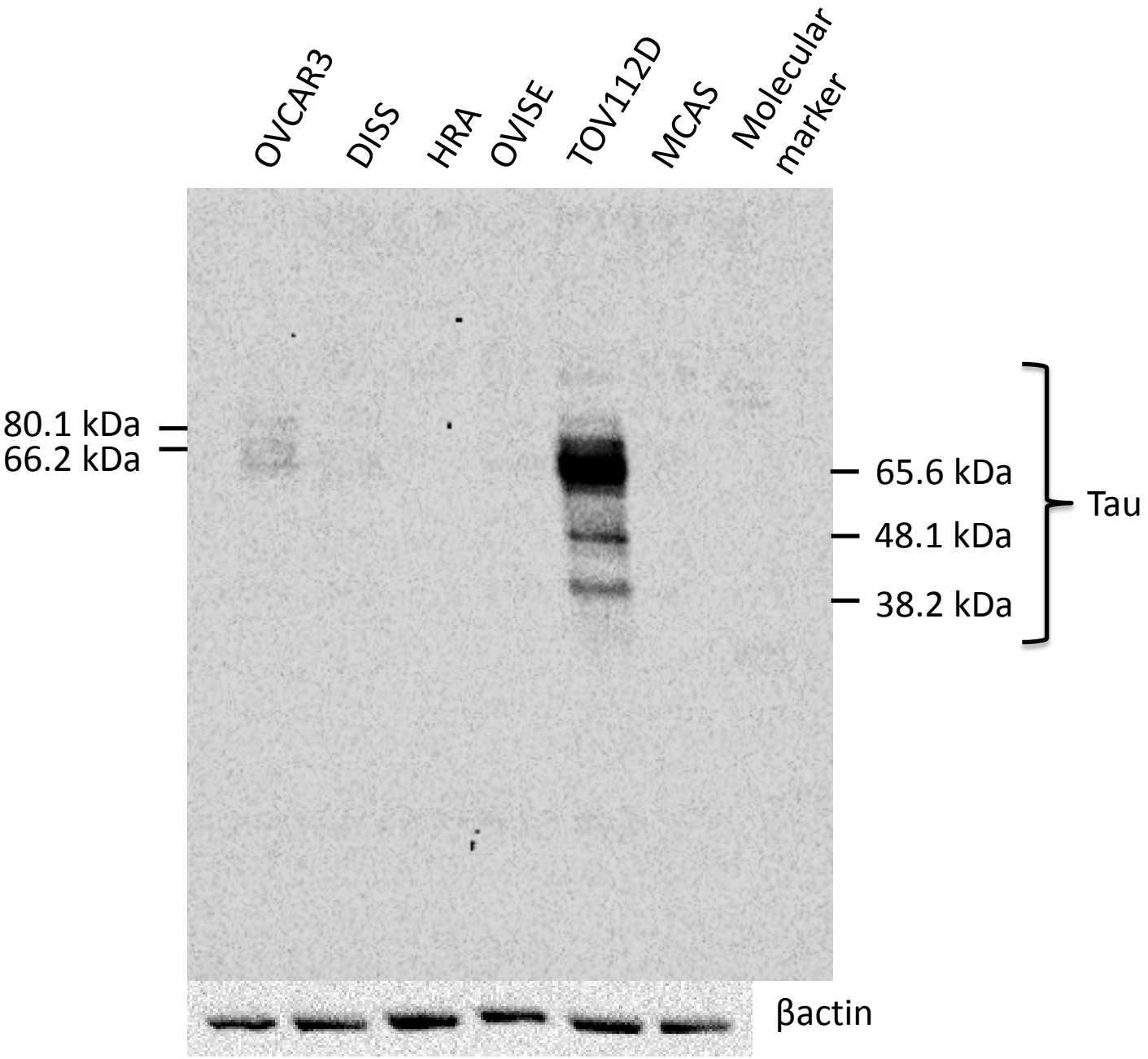
Figure 2. Alteration of tau protein expression after MAPT DNA or siRNA transfection. A) Comparison of the level of tau protein expression between control cells and MAPT DNA-transfected cells on western blot analysis. The expression of tau protein increased by the transfection of MAPT DNA in TOV112D cells and HRA cells. In particular, transfection of MAPT DNA showed multiple bands and its phenomenon suggested the hyper-phosphorylation. B) Comparison of the level of tau protein expression between control cells and MAPT siRNA-transfected cells on western blot analysis. The expression of tau protein decreased by the transfection of MAPT siRNA in TOV112D cells and OVCAR3 cells.

Figure 3. Cell proliferation assay. A) Cell proliferation of TOV112D cells transfected with MAPT DNA was significantly more inhibited compared with controls, at 0, 1, and 10 μg/ml paclitaxel. * $P < 0.05$. B) Cell proliferation of HRA cells transfected with MAPT DNA was not inhibited compared with controls, in almost all concentrations of paclitaxel.

Figure 4. Cell proliferation assay. A) Cell proliferation of TOV112D cells transfected with MAPT siRNA was significantly more inhibited compared with controls, at 0, 1, and 10 μg/ml paclitaxel. * $P < 0.05$. B) Cell proliferation of OVCAR-3 cells transfected with MAPT siRNA was not inhibited compared with controls, in almost all concentrations of paclitaxel.

Figure 5. Cell cycle analysis. The blue areas in the left part of the figures show the sub-G1 phase showing apoptosis. The average apoptosis rate was 4.1% for control cells and 29.0% for DNA-transfected cells. There is a significant increase in apoptosis of DNA-transfected cells. † $P < 0.001$ versus control. The average apoptosis rate was 11.7% for siRNA transfected-cells. There is a significant increase in apoptosis of MAPT siRNA-transfected cells. ‡ $P < 0.05$ versus control.

Figure 6. Apoptosis analysis. Annexin-V+/PI- cells show early apoptosis, and Annexin-V+/PI+ cells show late apoptosis. The average total (early + late) apoptosis rate of control cells was 12.8% (average early apoptosis, 11.3%; average late apoptosis, 1.5%). The average total apoptosis rate of DNA transfected-cells was 72.8% (average early apoptosis, 52.6%; average late apoptosis, 19.7%). There is a significant increase in apoptosis of DNA transfected-cells. § $P < 0.0001$ versus control. The average total apoptosis rate of siRNA transfected-cells was 29.9% (average early apoptosis, 27.6%; average late apoptosis, 2.3%). There is a significant increase in apoptosis of siRNA transfected-cells. # $P < 0.001$ versus control.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βactin

Figure 1

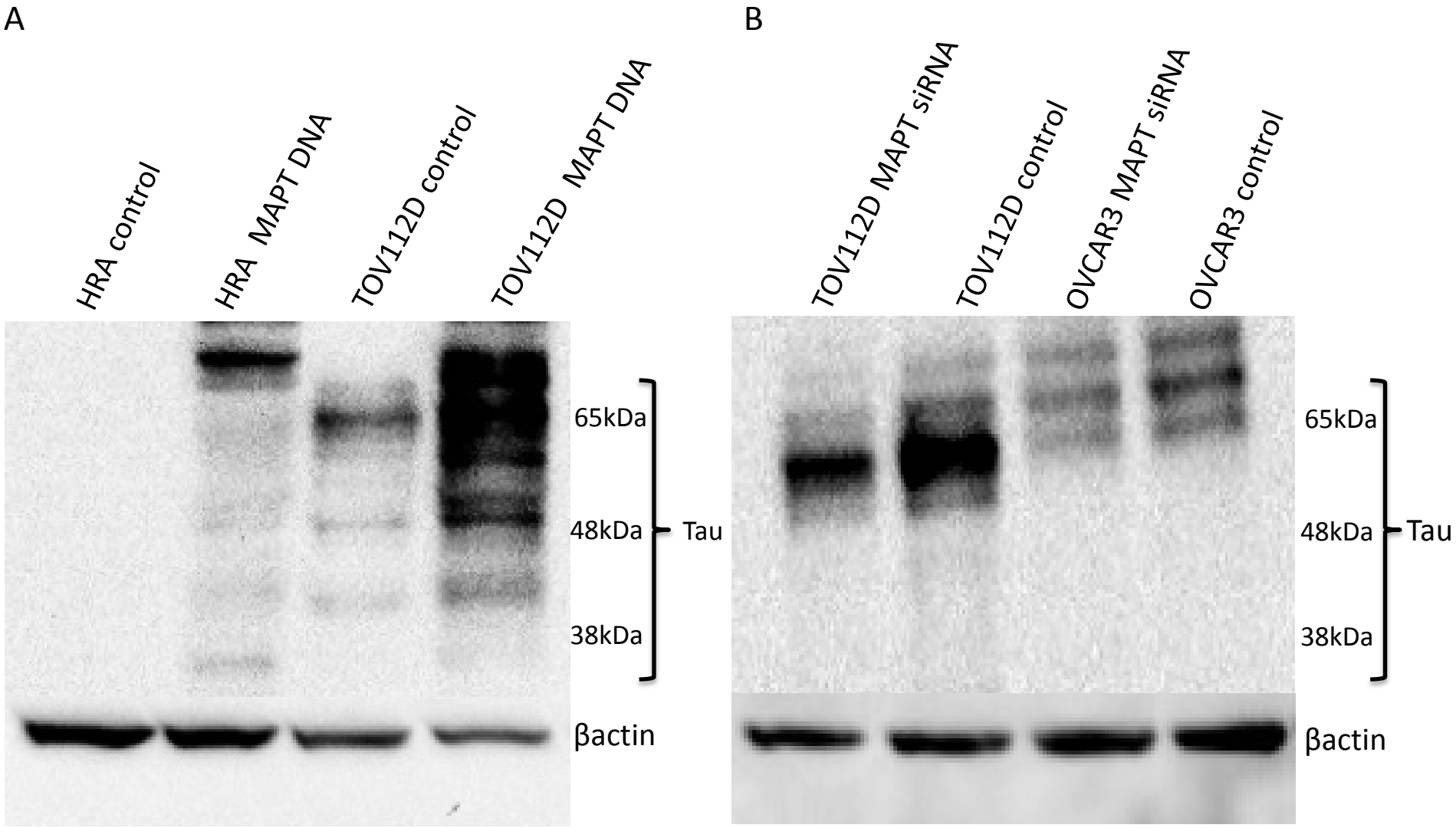
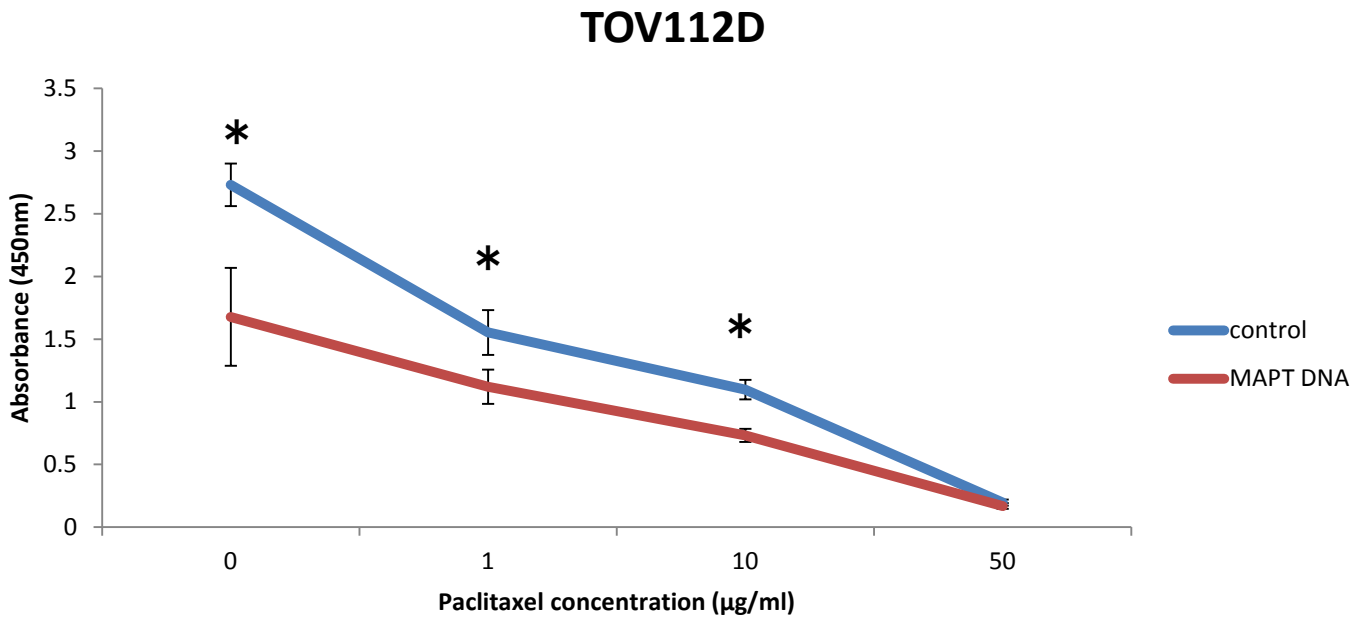

A
HRA control
HRA MAPT DNA
TOV112D control
TOV112D MAPT DNA
65kDa
48kDa
38kDa
Tau
βactin
B
TOV112D MAPT siRNA
TOV112D control
OVCAR3 MAPT siRNA
OVCAR3 control
65kDa
48kDa
38kDa
Tau
βactin

Figure 2

A


TOV112D
Absorbance (450nm)
3.5
3
2.5
2
1.5
1
0.5
0
*
*
*
0
1
10
50
Paclitaxel concentration (µg/ml)
control
MAPT DNA

B

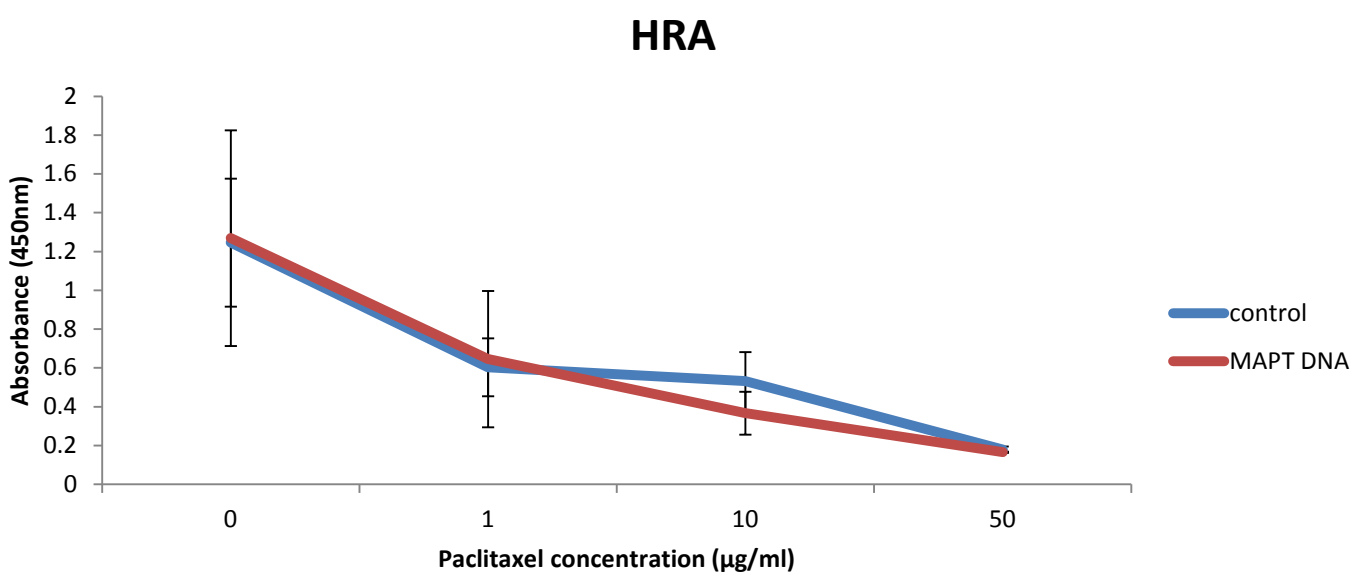
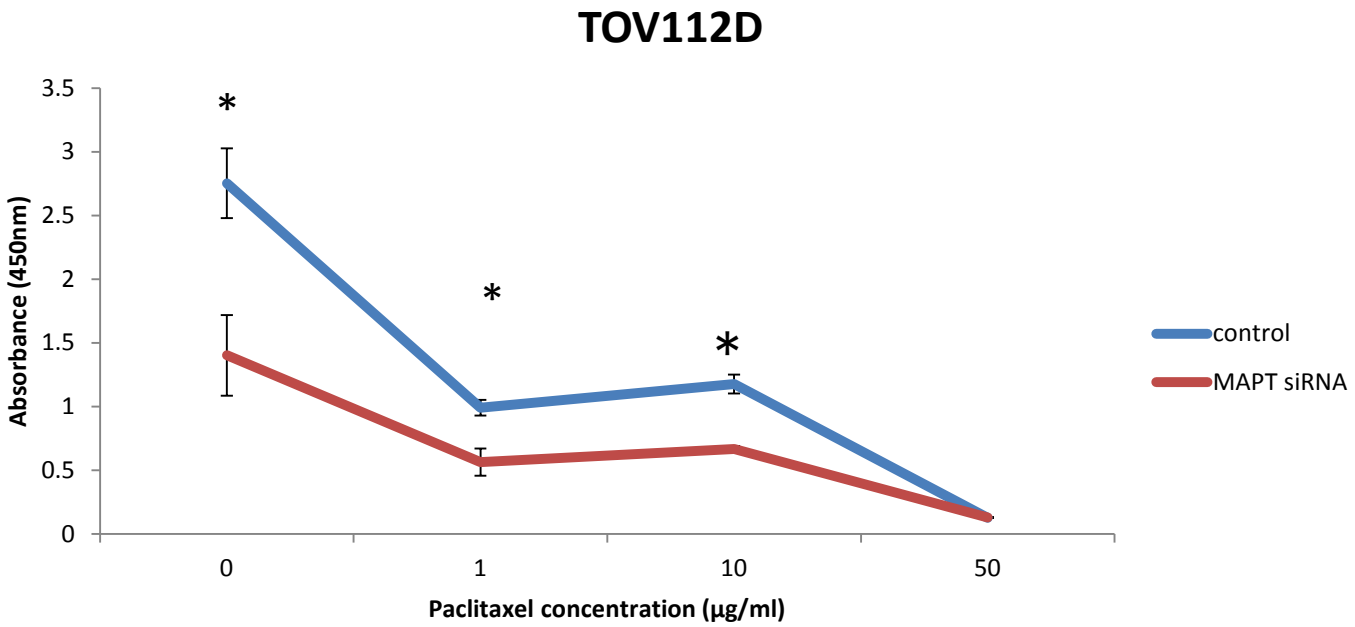

HRA
Absorbance (450nm)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
1
10
50
Paclitaxel concentration (µg/ml)
control
MAPT DNA

Figure 3

A


TOV112D
3.5
3
2.5
2
1.5
1
0.5
0
Absorbance (450nm)
*
*
*
0
1
10
50
Paclitaxel concentration (µg/ml)
control
MAPT siRNA

B

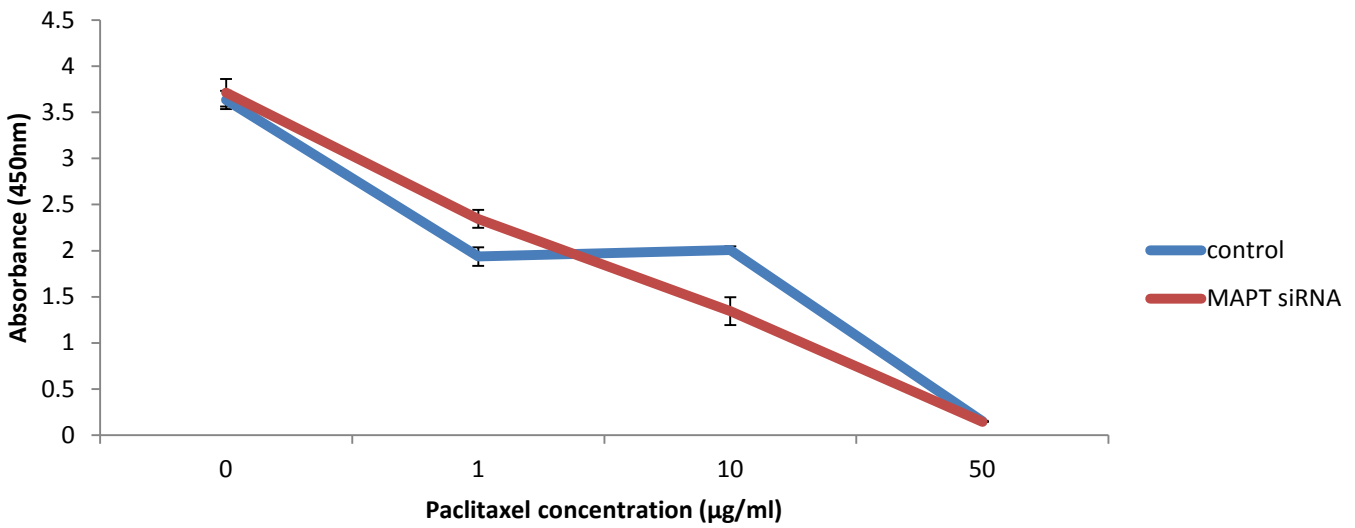
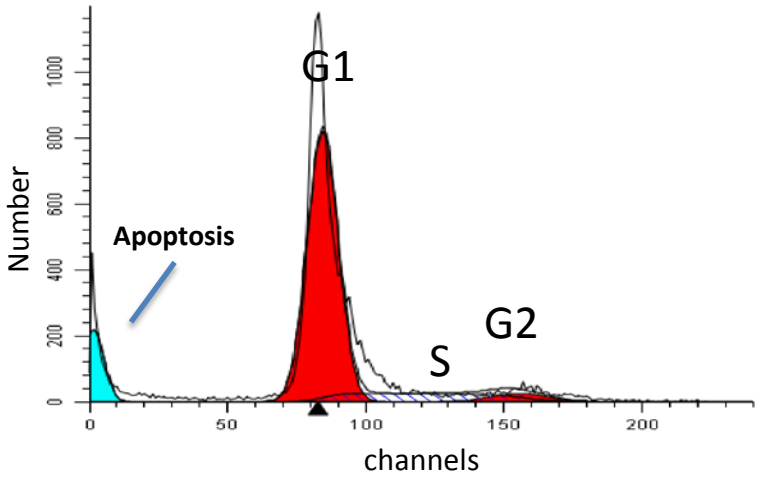
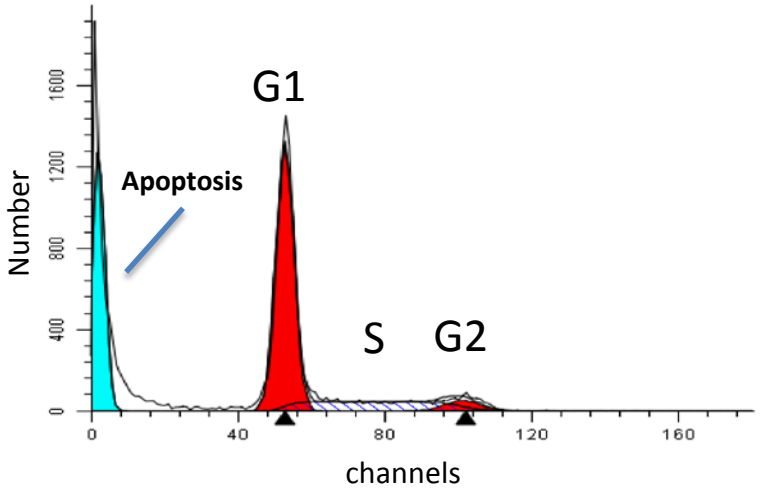

OVCAR3
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Absorbance (450nm)
0
1
10
50
Paclitaxel concentration (µg/ml)
control
MAPT siRNA

Figure 4

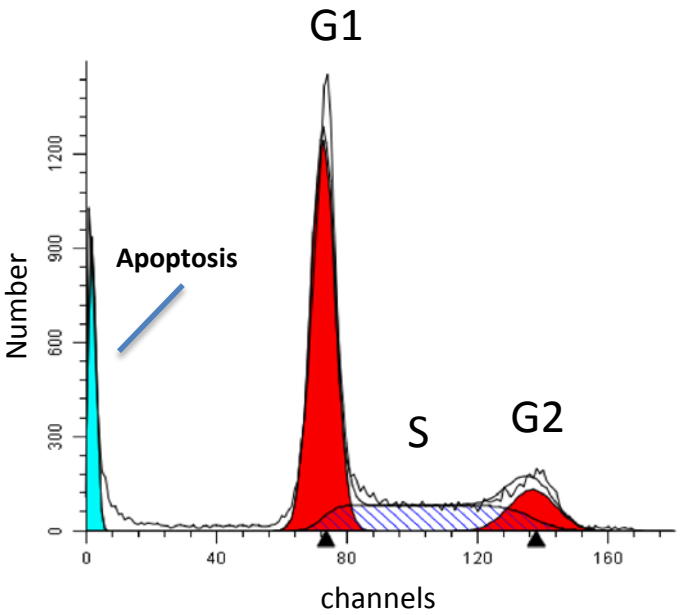
Control



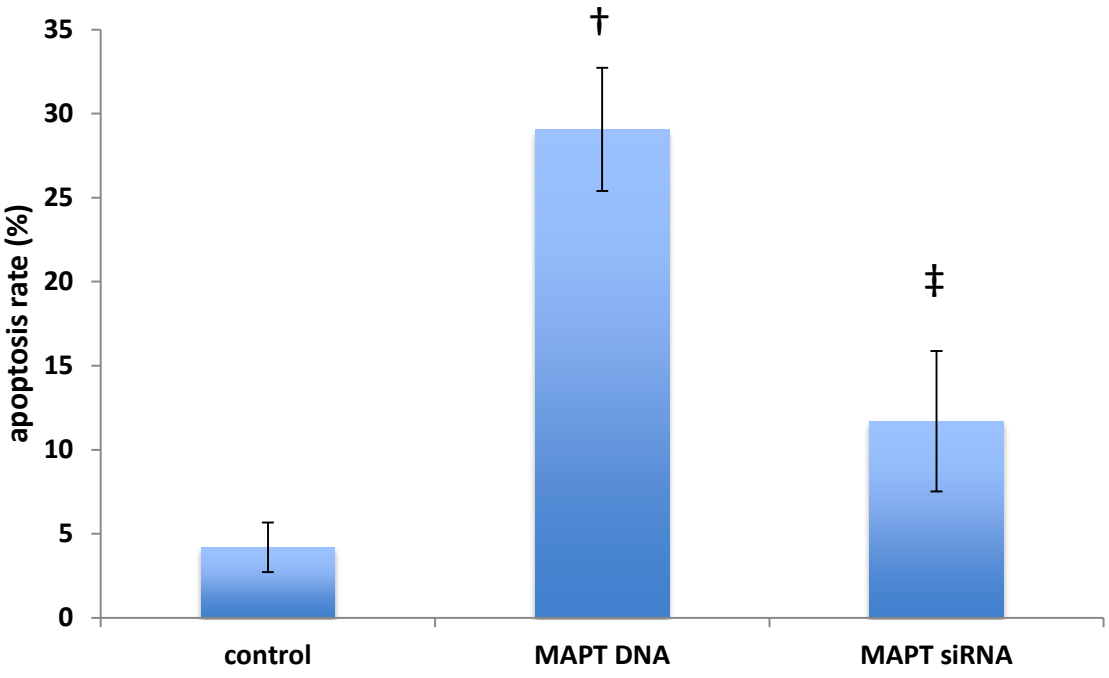
MAPT DNA transfection

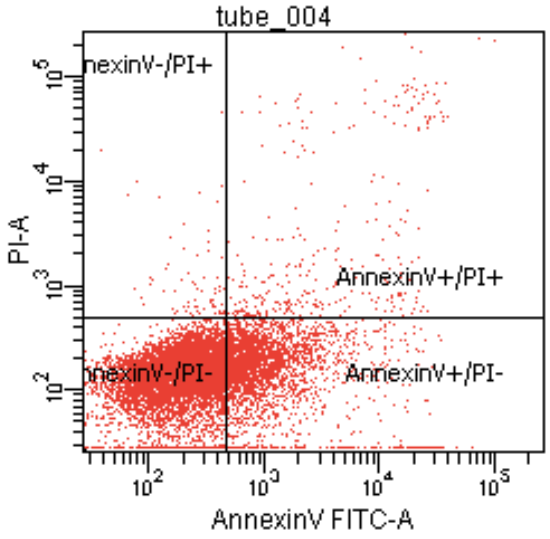


MAPT siRNA transfection

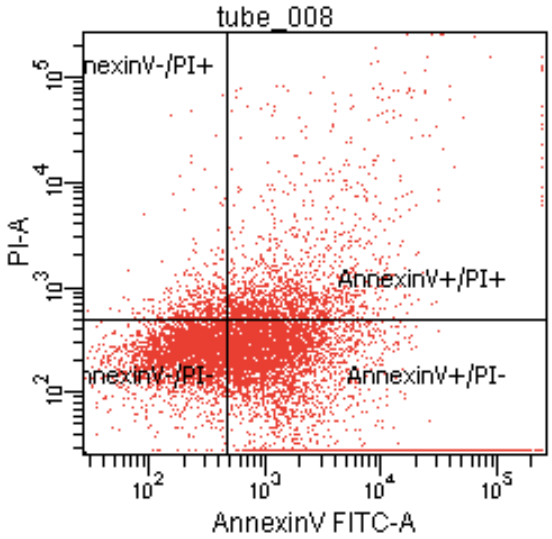


TOV112D cell cycle apoptosis rate

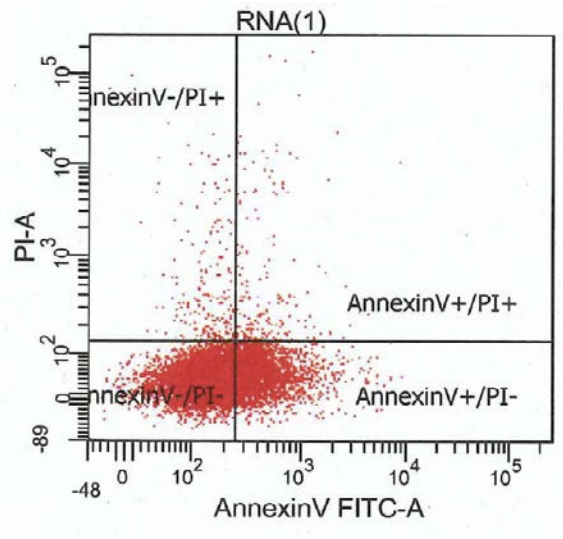




Control



MAPT DNA transfection



MAPT siRNA transfection

TOV112D apoptosis rate (early+late)

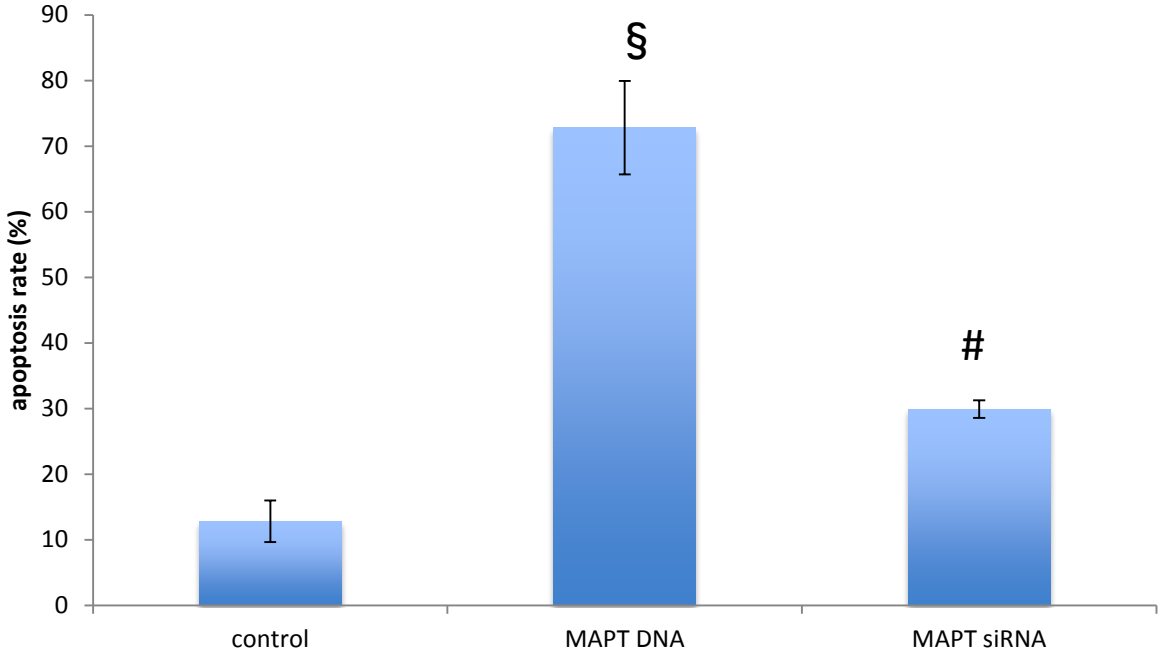


Figure 6