RESEARCH ARTICLE

Sensitization of Cervical Carcinoma Cells to Paclitaxel by an IPP5 Active Mutant

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Abstract

Paclitaxel is one of the best anticancer agents that has been isolated from plants, but its major disadvantage is its dose-limiting toxicity. In this study, we obtained evidence that the active mutant IPP5 (8-60hIPP5^m), the latest member of the inhibitory molecules for protein phosphatase 1, sensitizes human cervix carcinoma cells HeLa more efficiently to the therapeutic effects of paclitaxel. The combination of 8-60hIPP5^m with paclitaxel augmented anticancer effects as compared to paclitaxel alone as evidenced by reduced DNA synthesis and increased cytotoxicity in HeLa cells. Furthermore, our results revealed that 8-60hIPP5^m enhances paclitaxel-induced G2/M arrest and apoptosis, and augments paclitaxel-induced activation of caspases and release of cytochrome C. Evaluation of signaling pathways indicated that this synergism was in part related to down-regulation of NF-κB activation and serine/threonine kinase Akt pathways. We noted that 8-60hIPP5^m down-regulated the paclitaxel-induced NF-κB activation, IκBα degradation, PI3-K activity and phosphorylation of the serine/threonine kinase Akt, a survival signal which in many instances is regulated by NF-κB. Together, our observations indicate that paclitaxel in combination with 8-60hIPP5^m may provide a therapeutic advantage for the treatment of human cervical carcinoma

Keywords: IPP5 - paclitaxel - G2/M arrest - apoptosis - signal transduction - HeLa cells

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Introduction

Reversible protein phosphorylation regulates the biological activity of many protein complexes, and is regarded as a major mechanism for the control of cell cycle progression. It has been reported that the semi-selective inhibitors of PPases, such as okadaic acid, cantharidin, and fostriecin, influence several aspects of cell cycle progression (Cohen, 2002). An important Ser/Thr protein phosphatase, protein phosphatase-1 (PP1), regulates a series of physiological events, such as cell cycle, gene expression, protein synthesis, glycolipid metabolism and memory formation (Ceulemans and Bollen, 2004). Its critical function in mitosis is evidenced by the occurrence of metaphase arrest in various eukaryotic cells with PP1 mutation or inhibition (Booher and Beach, 1989; Kinoshita et al., 1990). The microtubule dynamics experiments also indicate that PP1 activity is necessary for the completion of mitosis (Cheng et al., 2000). Furthermore, abnormally high expression of PP1 was observed in some tumor cells, indicating that PP1 might promote the growth of malignant tumors (Sogawa et al., 1996). Protein phosphatase inhibitor-1 (PPI-1) is the first endogenetic molecule found to inhibit PP1 activity, when phosphorylated by protein kinase A (PKA) at Thr-35 (Nimmo and Cohen, 1978). However, when Thr-35 is mutated to Asp, PPI-1 could inhibit the activity of PP1 without phosphorylation by PKA. The active mutant of IPP5 (8-60hIPP5^m), the latest member of the inhibitory molecules for PP1, has been demonstrated to inhibit the activity of PP1 in Thr-40-dependent manner *in vitro* with a similar IC50 as PPI-1 (Wang et al., 2008). Previous studies from our laboratory have shown that 8-60hIPP5^m significantly inhibited the growth of human cervix carcinoma cells (HeLa) by inducing apoptosis (Zeng et al., 2009) and G2/M arrest (Zeng et al., 2012).

Carcinoma of the cervix is considered relatively resistant to chemotherapy. It is the second most common cancer in women worldwide and one of the most important causes of cancer-related death, especially in developing countries (Jemal et al., 2008). It has been known that the high prevalence of HPV infection occurs in cervical cancer, and the two commonest HPV genotypes in cervical cancer were HPV 16 and 18 (Wang et al, 2013). Despite the declining mortality rate for cervical cancer through the last decade, advanced or recurrent disease remains a major cause of death (Long, 2007). Current treatment modalities such as surgical ablation and/or external radiotherapy intervention remain largely palliative for cervical cancer patients because the disease recurs in a refractory form.

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Paclitaxel (Taxol) is capable of inhibiting microtubule depolymerization and arresting the cell cycle at the G2/M phase, which leads to apoptotic cell death (Horwitz, 1992). In the clinic, paclitaxel has been shown to have significant therapeutic benefits on ovarian, breast, non-small cell lung, and head and neck cancers (Sparano et al., 2008). However, the success of paclitaxel chemotherapy in cervical cancer patients is limited by the myelotoxicity and neurotoxicity (Zanetta et al., 2000). Furthermore, tumors tend to acquire resistance to cytotoxic chemotherapeutic agents, including paclitaxel (Koshiyama et al., 2006). The molecular basis of resistance to paclitaxel is not well understood. Paclitaxel has been implicated in regulating targeted cellular proteins that promote cell survival and block apoptosis (such as Bcl-2 and Bcl-XL) (Chun and Lee, 2004). Another mechanism of chemoresistance involves enhanced phosphorylation of protein kinase B/Akt (Page et al., 2000). Furthermore, NF-κB promotes cell survival and up-regulates genes which are important for tumor proliferation and metastasis (Pahl, 1999). So blocking NF-κB activation may augment cancer chemotherapy. Therefore, combination with agents that could inhibit agents that induce apoptosis and stimulate NF-κB activity may be effective.

Based on the finding that 8-60hIPP5^m induces apoptosis and G2/M arrest in HeLa cells, we hypothesized that it may sensitize cervical cancer cells to paclitaxel. In this study, we examined the effects of 8-60hIPP5^m overexpression in HeLa cells in the presence of paclitaxel, and found that the combination was very effective at inhibiting proliferation and inducing mitotic arrest and apoptosis. We also investigated the potential molecular mechanism underlying this synergistic effect, and determined whether the combination enhanced the inhibition of antiapoptotic signal transducers Akt and NF-κB, which leads to the increased activation of caspase-mediated apoptosis.

Materials and Methods

Reagents and cell culture

Human cervix carcinoma cells (HeLa) (ATCC, Manassas, VA, USA) were grown in DMEM medium supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS) (Hyclone, Logan, UT, USA), 4.5 g/liter D-glucose, nonessential amino acids (100 μM each), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine at 37°C in a 5% CO_2 incubator. The wild type human *IPP5* plasmid, *pcDNA3.1/myc-His (-)B-hIPP5* (referred to as *phIPP5-B* later), and active mutant hIPP5 (amino acids 8-60 of *hIPP5*, Thr→Asp-40) plasmid, *pcDNA3.1/myc-His (-)B-p8-60hIPP5^m* (referred to as *p8-60hIPP5^m*-B later) were kindly provided by Dr. Xiao-Jian Wang at Zhejiang University (Zhejiang, China).

Cell transfection

The expression vectors *phIPP5-B* and *p8-60hIPP5^m-B* were transfected into HeLa cells using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) with *pcDNA3.1/myc-His (-)B* as a mock control. Stable cell lines overexpressing hIPP5 or 8-60hIPP5^m were selected by 600-1000 μg/ml G418 for 2-3 weeks, and cloned by limiting dilution. These stable cell lines were designated as *HeLa-hIPP5* and *HeLa-8-60hIPP5^m*, respectively. The established stable cell lines were maintained in the same culture medium as used for parental HeLa cells. The stable expression of hIPP5 or 8-60hIPP5^m was confirmed by RT-PCR and Western blot. The stably transfected cells were treated with 10 nM paclitaxel (Concord Pharmaceutical, China) for different periods of time and then subjected to Western blot analysis, DNA analysis, and apoptosis assay.

[^{3}H]Thymidine incorporation

HeLa-hIPP5, *HeLa-8-60hIPP5^m*, HeLa-mock or parental HeLa cells (5×10^3/well) were seeded into 96-well plates and cultured in 10% FCS-DMEM containing 0.05 nM paclitaxel for 72 h. After removing the medium, [^{3}H] Thymidine (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK) at a dose of 0.5 Ci/well was added, and the cells were cultured in10% FCS-DMEM for another 18 h. Then the cells were harvested onto glass fibers with a multiple cell harvester, and the proliferation of HeLa cells was assessed by [^{3}H] thymidine incorporation using a β-Scintillation Counter (Wallac, Milton Keynes, Bucks, UK). Results of [^{3}H] thymidine incorporation (cpm) were expressed as means±SD.

Colony formation in soft agar

Single-cell suspensions of *HeLa-hIPP5*, *HeLa-8-60hIPP5^m*, HeLa-mock or parental HeLa cells (500 cells) were cultured using 0.3% type II agarose in 10% FCS-DMEM containing 0.05 nM paclitaxel on 6-well plates that were previously coated with 0.6% type II agarose and incubated under standard culture conditions. After 7-10 days, the numbers of colonies (>50 cells) were counted under an inverted microscope.

Cell cycle analysis

Cells were harvested and washed in PBS, then fixed in 75% alcohol for 30 min at 4°C. After washing in cold PBS for three times, cells were resuspended and incubated in 1 ml of PBS containing 40 μg of propidium iodide (PI, Sigma Chemical Co., St.Louis, MO, USA) and 100 μg of RNase A (Sigma Chemical Co.) for 30 min at 37°C. Samples were then analyzed for DNA contents using a fluorescence-activated cell sorter FACSCalibur (Becton Dickinson, Mountain View, CA, USA).

Apoptosis assay

Cells were exposed to 10 nM paclitaxel for 24 h, harvested, and stained with PI, Rhodamine 123 (R-302, Molecular Probes, Eugene, OR, USA) according to the manufacturer's instructions. Stained cells were analyzed using FACSCalibur.

Assay of caspases

After treatment with 10 nM paclitaxel for 8 h, cells were collected and lysed. Caspase-3 activity was assayed using Caspase-3 Colorimetric Assay kit (BD Pharmingen, San Diego, CA, USA), and caspase-8 activity was assayed using Caspase-8 Activity Assay kit (Chemicon International, Temecula, CA, USA) according to manufacturers' instructions.

Western blot analysis

The protein concentrations were determined using the BCA Protein Assay kit (Pierce Biotechnology, Rockford, IL, USA). Samples containing equal amounts of protein were separated by 12% SDS-PAGE, and electrophoretically transferred to Protran nitrocellulose membranes (Schleicher & Schuell, Keene, NH, USA). Membranes were blocked with TBST containing 10% nonfat milk before incubating with primary antibodies specific for Myc, β-actin, cyto-chrome c, NF-κB p65 (sc-109), nucleoporin p62, Akt, phospho-Akt (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), IκB-α (Biolabs, Beverly, MA, USA), respectively. After washing, the membranes were incubated with secondary antibodies conjugated with horseradish peroxidase (HRP) (Cell Signaling Technology Inc., Beverly, MA, USA). The HRP activity was detected using SuperSignal West Femto Maximum Sensitivity Substrate (Pierce Biotechnology).

Measurement of cytochrome c release

Cells were lysed in lysis buffer (10 mM HEPES [N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pH 7.5], 10 mM KCl, and 1 mM EDTA [ethylenediaminetetraacetic acid]) supplemented with protease inhibitor cocktail (Sigma). The cells in lysis buffer were frozen and thawed 3 times, and spun at 2000g for 5 min. The supernatant was further centrifuged at 60 000g for 30 min at 4°C, and used for cytochrome c content analysis by Western blot.

Assessment of IκBa degradation and NF-κB nuclear translocation

Cytoplasmic and nuclear extracts were prepared using NE-PER nuclear and cytoplasmic extraction reagents (Pierce Biotechnology, Rockford, IL, USA), and the protein concentrations were determined using the BCA protein assay kit. IκBα in cytoplasmic extracts and NF-κB subunit p65 in nuclear extracts were detected by Western blot using specific antibodies.

Assay of PI3-K activity

PI3-K activity was assayed with a PI3-K ELISA kit (Echelon Biosciences Inc., Salt Lake City, UT, USA) according to the manufacturer's instructions. In brief, cells were treated with 10 nM paclitaxel for 1 h. Cell lysates were prepared, and PI3-K protein was immunoprecipitated with an antibody against the p85 subunit and incubated with PI (4, 5)P2. The reaction products were incubated with a PI (3, 4, 5)P3 detector protein before adding to PI (3, 4, 5)P3-coated microplates for competitive binding. A peroxidase-linked secondary antibody and colorimetric detection reagents were used to detect PI (3, 4, 5)P3 detector protein binding to the plate. The colorimetric signal was inversely proportional to the amount of PI (3, 4, 5)P3 produced by PI3-K activity.

Statistical analysis

All experiments were repeated a minimum of three times. Pairwise comparisons were conducted using Student's t test. *p* values of less than 0.05 were considered statistically significant.

Results

8-60hIPP5^m enhances the inhibition of cell proliferation by paclitaxel

To investigate the biological functions of human IPP5, human cervical carcinoma HeLa cells were transfected with hIPP5 or 8-60hIPP5^m expression vector. We have previously demonstrated that hIPP5 or 8-60hIPP5^m gene can be efficiently transfected into HeLa cells by RT-PCR and Western blot (Zeng et al., 2009). In order to investigate whether there is any synergism between paclitaxel and 8-60hIPP5^m, [3H] thymidine incorporation was used to evaluate the DNA synthesis, which reflects the cell proliferation. The result showed that overexpression of 8-60hIPP5^m enhanced the inhibition of HeLa cell proliferation by paclitaxel. As shown in Figure 1a, when cells were treated with paclitaxel, [3H] thymidine incorporation level in *HeLa-8-60hIPP5^m* cells was about 25% of that in mock-HeLa cells ($p < 0.01$), which had a similar proliferative rate as parental HeLa cells. We also performed colony formation assays and found that clonal growth of *HeLa-8-60hIPP5^m* cells was also significantly inhibited compared to mock-HeLa cells or parental HeLa cells in the culture medium containing 0.05 nM paclitaxel (Figure 1b). Taken together, these results suggest that 8-60hIPP5^m synergizes with paclitaxel to inhibit HeLa cell proliferation.

8-60hIPP5^m synergizes with paclitaxel to induce apoptosis

We further investigated whether 8-60hIPP5^m could

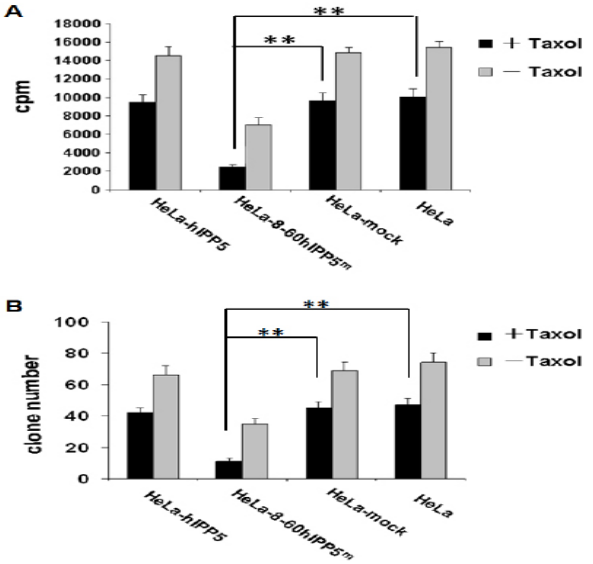

Figure 1. 8-60hIPP5^m Enhances the Inhibition of Cell Proliferation by Paclitaxel. a, Stably transfected HeLa cells and parental HeLa cells were treated with paclitaxel as indicated. Cell proliferation was determined by (3H) thymidine incorporation assay. Values shown are means±SD of quadruplicate cultures from one experiment, which is representative of four independent experiments conducted. b, Stably transfected HeLa cells and parental HeLa cells (5×10^3/well) were cultured in type II agarose medium containing 0.05 nM paclitaxel. Colonies (>50 cells) were counted after 7 days of incubation. Values are expressed as means±S.D. of triplicate cultures. **, $p<0.01$ versus paclitaxel-treated parental HeLa cells or paclitaxel-treated HeLa-mock cells

enhance the ability of paclitaxel to induce apoptosis. Figure 2 showed that the combination of 8-60hIPP5^m with paclitaxel increased apoptosis to 48.82%, which is significantly higher than 8-60hIPP5^m or paclitaxel alone (p <0.01). Thus, although 8-60hIPP5^m had some effect on its own, it greatly enhanced the ability of paclitaxel to induce apoptosis in HeLa cells.

One mechanism of apoptosis is related to the activation of the caspase cascade. So we examined caspase-3 and caspase-8 activities in paclitaxel-treated cells. As shown in Figure 3a, treatment of *HeLa-8-60hIPP5^m* cells with paclitaxel significantly increased caspase-3 activity. Caspase-8 activity was slightly higher in *HeLa-8-60hIPP5^m* cells treated with paclitaxel, but the difference was not statistically significant. The activation of caspase-3 by paclitaxel in *HeLa-8-60hIPP5^m* cells suggests that the apoptosis could be mediated, at least in part, through cytochrome c release from the mitochondria in these cells. To test this hypothesis, we examined the effects of 8-60hIPP5^m and paclitaxel treatments on cythochrome c release. Figure 3b showed that 8-60hIPP5^m or paclitaxel alone did not affect the accumulation of cytochrome c in the cytosol. However, the combination of 8-60hIPP5^m and paclitaxel resulted in a significant increase of cytochrome c release. Cytochrome c release to the cytoplasm could be resulted from an increase in proapoptotic Bcl2 family members or a decrease in prosurvival Bcl2 family members.

8-60hIPP5^m synergizes with paclitaxel to induce G2/M phase arrest

Paclitaxel stabilizes microtubules and causes mitotic arrest, which is important to paclitaxel-induced apoptosis (Woods et al., 1995; Jordan et al., 1996). We examined the effect of combination of both 8-60hIPP5^m and paclitaxel on cell-cycle progression. As shown in Figure 4, 8-60hIPP5^m or paclitaxel alone induced G2/M arrest to some degree respectively. However, the combination

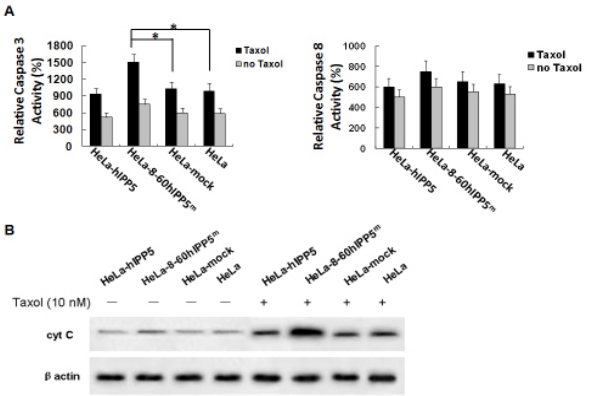

Figure 3. 8-60hIPP5^m Synergizes with Paclitaxel to Induce Cytochrome c (cyt C) Release and Caspase-3 Activation. a, 8-60hIPP5^m synergized with paclitaxel to activate caspase-3. Stably transfected HeLa cells were treated with paclitaxel (10 nM) for 24 h before caspase-3 and caspase-8 activity assays. *p<0.05 versus paclitaxel-treated parental HeLa cells or paclitaxel-treated HeLa-mock cells. b, 8-60hIPP5^m synergized with paclitaxel to induce cytochrome c (cyt C) release. Stably transfected HeLa cells were treated with paclitaxel (10 nM) for 24 h before Western blot analysis

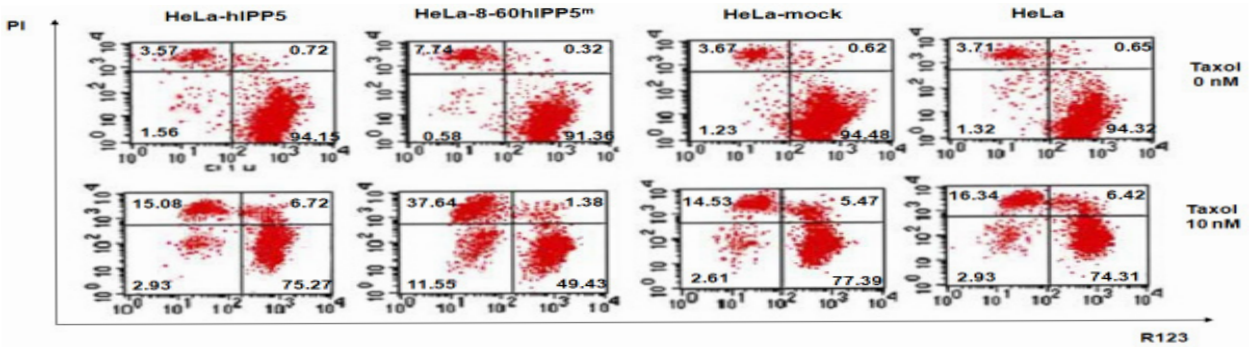

Figure 2. 8-60hIPP5^m Synergizes with Paclitaxel to Induce Apoptosis. Stably transfected HeLa cells were treated with paclitaxel (10 nM) for 24 h. Cells were labeled by green fluorescent cationic dye (Rhodamine 123, R-123) and PI. The percentages of lower left and upper left represent the early and the late apoptotic cells, respectively

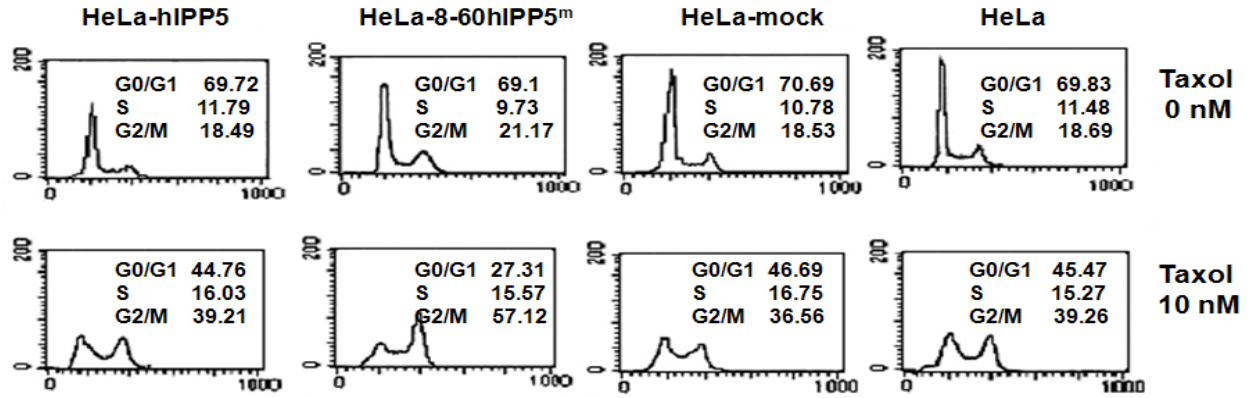

Figure 4. 8-60hIPP5^m Synergizes with Paclitaxel to Induce G2/M Arrest. Stably transfected HeLa cells were treated with paclitaxel (10 nM) for 8 h before PI staining and flow cytometry analysis

of 8-60hIPP5^m with paclitaxel significantly decreased the proportion of HeLa cells in the G0/G1 phase (from 69.83% to 27.31%, $p<0.01$) and increased the proportion of cells in the G2/M phase (from 18.69% to 57.12%, $p<0.01$).

Paclitaxel-induced NF-κB activation and IκBα degradation are inhibited by 8-60hIPP5^m

Under normal conditions, the majority of NF-κB subunits are sequestered in the cytoplasm by I-κBα, and translocated into the nucleus following I-κBα degradation. Paclitaxel activates NF-κB in several cell lines through the degradation of IκBα (Das and White, 1997; Lee and Jeon, 2001; Smitha et al, 2005). To investigate whether paclitaxel- induced NF-κB activation is through I-κBα degradation, western blot was performed against anti-IκBα using the cytoplasmic protein extracts. As shown in Figure 5, we observed the activation of NF-κB in HeLa cells by paclitaxel, and noticed that 8-60hIPP5^m down-regulated the nuclear accumulation of NF-κB p65 subunit induced by paclitaxel. The nuclear extract of HeLa cells treated with tumor necrosis factor-α, a well known activator of NF-κB, was used as the positive control. Interestingly, consistent with the inhibition of NF-κB activation in *HeLa-8-60hIPP5^m* cells in response to paclitaxel, the total I-κBα level in paclitaxel-treated *HeLa-8-60hIPP5^m* cells showed no significant change while that of paclitaxel-treated control cells was decreased, indicating that 8-60hIPP5^m could inhibit paclitaxel-induced I-κBα degradation in HeLa cells. These results suggest that 8-60hIPP5^m inhibits NF-κB activation likely through blocking the degradation and/or inducing the synthesis of the inhibitory protein IκBα.

8-60hIPP5^m inhibits paclitaxel-induced PI3-K/Akt activation

Akt is a survival signal mediator that in many cases is regulated by NF-κB (Ozes et al., 1999; Pianetti et al., 2001). As shown in Figure 6a, after treatment with paclitaxel, Akt phosphorylation was clearly observed in control cells, while 8-60hIPP5^m almost completely abolished the phosphorylation of Akt, indicating a possible role for Akt in the synergistic effect of paclitaxel and

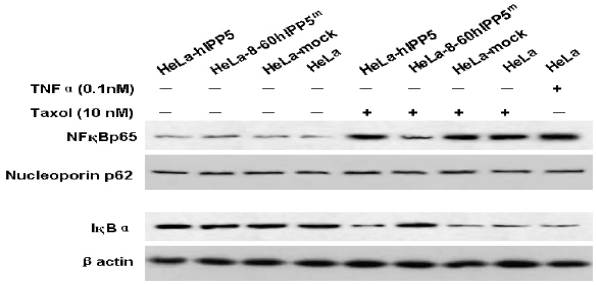

Figure 5. 8-60hIPP5^m Inhibits Paclitaxel-Induced NF-κB Activation and IκBα Degradation. Stably transfected HeLa cells in 6-well plates were treated with paclitaxel (10 nM) for 60 min. Tumor necrosis factor (0.1 nM) treatment was used as a positive control. Cells were collected and proteins were extracted using NE-PER® Nuclear and Cytoplasmic Extraction Reagents. NF- κB p65 in nuclear extract and IκB- α in cytosol were detected by Western blot. Nucleoporin p62 and actin were used as the nuclear and cytosol protein loading controls, respectively

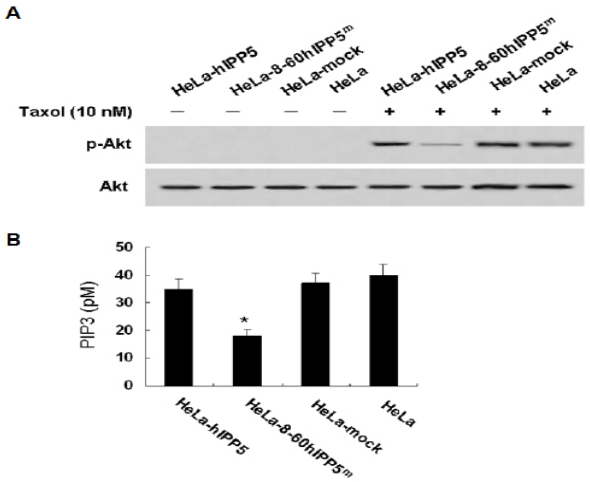

Figure 6. 8-60hIPP5^m Inhibits Paclitaxel-Induced PI3-K/Akt Activation. a, paclitaxel-induced Akt activation is down-regulated by 8-60hIPP5^m in HeLa cells. Stably transfected HeLa cells were treated with paclitaxel (10 nM) for 1 h. Whole cell lysates were resolved on a 10% PAGE gel and blotted against phospho-Akt serine 473. b, 8-60hIPP5^m inhibits paclitaxel-induced PI3-K activity. Stably transfected HeLa cells were treated with paclitaxel (10 nM) for 1h. Cell lysates were prepared for PI3-K activity assay. Similar results were obtained in three separate experiments. *$p<0.05$ versus parental HeLa cells or HeLa-mock cells

8-60hIPP5^m. Similarly, 8-60hIPP5^m decreased PI3-K activity in paclitaxel-treated cells (Figure 6b). Taken together, these results suggest that the enhancement of paclitaxel-induced apoptosis by 8-60hIPP5^m is likely through the inhibition of NF-κB activation, IκBα degradation, and PI3-K/Akt activity, resulting in the chemosensitization of HeLa cells to paclitaxel.

Discussion

Cervical cancer is one of the common gynecologic malignancies. Recent studies reported that docetaxel and cisplatin in concurrent chemoradiotherapy in advanced cervical cancer has a good short-term effect, but increased the toxicity, so the long-term effect needs further observation (Ke et al., 2012). Ionizing radiation can induce many base alteration, so increasing the sensitivity of tumor cells to chemotherapy would improve outcome in patients with cervical cancer.

Protein phosphatase 1 (PP1) is a major eukaryotic protein serine/ threonine phosphatase that regulates a variety of cellular functions through the interaction of its catalytic subunit with different regulatory subunits. IPP5, the latest member of PP1 inhibitory molecules, contains 116 amino acids. IPP5 shares significant homology to IPP1, especially in the two conserved motifs of the N terminus, KIQF and Thr-40. 8-60hIPP5^m, the constitutively activated form of hIPP5, which contains 8-60 residues in the N terminus, with an Asp substituting for Thr-40 to mimic the functional effects of phosphorylation, could inhibit PP1 activity without phosphorylation by PKA.

In this study, we found that 8-60hIPP5^m could synergize with paclitaxel to arrest human cervical cancer cells at G2/M phase. We also found that 8-60hIPP5^m

sensitized human cervical cancer cells to paclitaxel-induced apoptosis likely through the inhibition of NF-κB activation, IκBα degradation, and PI3-K/Akt activity. This study is an extension of our earlier work in which it was discovered that 8-60hIPP5^m caused HeLa cell growth retardation *in vitro* and *in vivo* by inducing G2/M arrest and apoptosis (Zeng et al., 2009; 2012).

In eukaryotic organisms, cell cycle progression is regulated to a large extent by the reversible phosphorylation of various proteins. PP1 plays an important role during cell cycle progression, especially during mitosis. Accumulating evidences demonstrate that both phosphorylation and dephosohprylation are involved in the control of cell cycle. Neutralizing PP1 by anti-PP1 antibodies, mutation of PP1, or treatment with various natural phosphatase inhibitors (such as okadaic acid, calyculin-A, tautomycin, microcystin-LR, fostriecin and cantharidin) have been shown to interfere with cell cycle progression and checkpoint abrogation, resulting in multiple aberrant mitotic spindles and apoptotic cell death (Kinoshita et al., 1990; Van Dolah and Ramsdell, 1992; Sakoff et al., 2002). In human cells, PP1 could act as a histone H1 phosphatase, which is required for chromatin decondensation during the exit from mitosis (Paulson et al., 1996). The subcellular localizations of PP1 isoforms change at mitosis. PP1α is located at the centrosome, while PP1γ and PP1δ are associated with mitotic spindles and chromosomes, respectively (Andreassen et al., 1998). This might explain why mutation or inhibition of PP1 can cause complex abnormal phenotypes, including delayed transition of metaphase to anaphase, condensed chromosomes, formation of abnormal spindles, microtubule dynamics and chromosome separation malfunction, and defect in cytokinesis.

One of the mechanisms of paclitaxel-induced G2/M arrest is that paclitaxel can induce CDK1 activation, inhibit the expression of cyclin A and cyclin B1 proteins in a time-dependent manner, and up-regulate the Cdk inhibitor, p21WAF1/CIP1 (Choi and Yoo, 2012). Our previous research also demonstrated that 8-60hIPP5^m can induce CDK1 activation in HeLa cells transfected with 8-60hIPP5^m, delay the expression of cyclin A and cyclin B1 in the cell cycle, and up-regulate p21WAF1/CIP1 expression (Zeng et al., 2012). These findings suggest that CDK1, cyclin A, cyclin B1 and p21WAF1/CIP1 may be the common target molecule for paclitaxel and 8-60hIPP5^m, and both paclitaxel and 8-60hIPP5^m have the same effects on these molecules. These may help to explain why 8-60hIPP5^m can enhance G2/M arrest by paclitaxel. However, further research is needed to investigate the exact mechanism about how 8-60hIPP5^m synergizes with paclitaxel to induce G2/M arrest.

Tumor cells often evade apoptosis by overexpressing antiapoptotic proteins such as Bcl-2, NF-κB, and Akt, which provide them with survival advantages (Wang et al., 1999; Vivanco and Sawyers, 2002). Paclitaxel activates NF-κB in several cell systems, probably through the principal kinase IKK-β (Lee and Jeon, 2001). In the present study we observed that paclitaxel-induced NF-κB activation was down-regulated in *HeLa-8-60hIPP5^m* cells, which may contribute to the sensitization of *HeLa-8-60hIPP5^m* cells to paclitaxel-induced apoptosis. Studies published previously describe paclitaxel as an activator of Akt, which is a serine/threonine protein kinase and a downstream target of phosphoinositide 3-kinase (Mabuchi, 2002). We observed that paclitaxel-induced Akt activation was suppressed in *HeLa-8-60hIPP5^m* cells. It is known that Akt suppresses apoptosis by activating NF-κB (Ozes et al., 1999; Pianetti et al., 2001). According to a recent report, treatment with LY294002, a specific inhibitor of phosphoinositide 3-kinase, resulted in the enhancement of paclitaxel-induced cytotoxicity. This process was followed by the inhibition of NF-κB transcriptional activity, indicating that NF-κB may be the crucial intermediary mediator connecting Akt with the intrinsic susceptibility of cancer cells to chemotherapeutic agents (Nguyen et al., 2004).

In conclusion, we report a novel role for 8-60hIPP5^m in sensitizing cervical carcinoma cells to paclitaxel. We found that 8-60hIPP5^m synergizes with paclitaxel to induce G2/M arrest and apoptosis, which may involve the inhibition of NF-κB activation, IκBα degradation, and PI3-K/Akt activity. These results suggest that 8-60hIPP5^m might be explored for therapeutic uses in combination with chemotherapy.

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