**Advances in Brief**

**Inactivation of the Antiapoptotic Phosphatidylinositol 3-Kinase-Akt Pathway by the Combined Treatment of Taxol and Mitogen-activated Protein Kinase Kinase Inhibition**

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**Abstract**

Paclitaxel (Taxol) activates a number of signal transduction pathways that lead to apoptosis. In contrast, paclitaxel also activates cell survival pathways, such as the Raf-mitogen-activated protein kinase kinase (MEK)-extracellular signal-regulated kinase (ERK) pathway. Previously, we have shown that inhibition of MEK combined with paclitaxel treatment causes an impressive enhancement of apoptosis in various tumor cell lines. Here, we find that the combination of paclitaxel with a MEK inhibitor leads to a dramatic inactivation of the antiapoptotic Akt (protein kinase B) kinase. The decrease in Akt is not reflected at the protein or mRNA level but rather attributed to kinase inactivation. To confirm that inactivation of Akt is significant, a constitutively active Akt mutant was introduced and shown to reverse tumor cell apoptosis. Further analysis upstream of Akt shows that treatment with the combination of paclitaxel and MEK inhibitor down-regulates PI3K activity more than either agent alone. The direct pharmacological inhibition of phosphatidylinositol 3-kinase (PI3K) similarly enhances paclitaxel-induced tumor apoptosis in a dose-dependent manner. Our results suggest the combination of paclitaxel and MEK inhibitor leads to down-regulation of the PI3K-Akt signaling in addition to the proapoptotic effects of paclitaxel and MEK inhibitor alone. Overall, these findings render the combined use of paclitaxel with MEK inhibitors, or paclitaxel with PI3K inhibitors, as a promising new strategy for cancer chemotherapy.

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**Introduction**

The predominant mode of action of paclitaxel (Taxol) is the binding to β-tubulin, stabilizing the microtubule and preventing its depolymerization (1–3). Additionally, paclitaxel activates signal transduction pathways leading to gene expression. Paclitaxel has been shown to alter signal transduction cascades leading to the gene expression and production of several different cytokines (4–9). Paclitaxel also activates signaling cascades involved in apoptosis, such as JNK, \(^3\)Raf-1, and Bcl-2 family members (10–12). Most relevant to this work, paclitaxel activation of the JNK MAP kinase pathway has been shown to be important in paclitaxel-induced apoptosis (13–15). In contrast, paclitaxel also causes the activation of the MEK-ERK pathway, which is considered a proliferation and cell survival pathway.

Chemotherapy-induced activation of cell survival pathways is increasingly observed for conventional anticancer drugs, and targeting these survival signals will be invaluable for the design of rational and novel combination therapies. For example, the chemotherapeutic compounds etoposide, daunorubicin, and camptothecin enhance NF-κB activity, which promotes cell survival and chemoresistance (16–18). In these cases, the ablation of NF-κB greatly enhanced tumor cell death. Moreover, the MAP kinase family members JNK, ERK, and p38 are also activated in response to a wide variety of extracellular stimuli. Earlier reports have demonstrated that a delicate balance between JNK and ERK activation exists in determining neuronal cell death or survival in response to growth factors (19, 20). The balance between JNK and ERK activation is equally important in cancer and drug-induced apoptosis. Previously, we have shown that a combination of low doses of paclitaxel (10 nm) that activates proapoptotic JNK and the small molecule MEK inhibitors (U0126 or PD98059), which inhibit ERK1/2, causes a dramatic increase in tumor cell apoptosis (21). Additional detailed reports have also shown that the inhibition of the MEK-ERK pathway in combination with paclitaxel enhances tumor cell apoptosis (21–23).

In the ERK MAP kinase cascade, activated Raf-1, a serine-threonine kinase, initiates the signaling cascade through MEK, which in turn phosphorylates a second serine-threonine kinase ERK. ERK phosphorylates additional kinases and specific transcription factors important in cell proliferation and survival. In

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3 The abbreviations used are: JNK, c-Jun NH\(_2\)-terminal kinase; MAP, mitogen-activated protein; MEK, MAP kinase kinase; ERK, extracellular signal-regulated kinase (ERK); PI3K, phosphatidylinositol 3-kinase; PIP, phosphatidylinositol phosphate; PH, pleckstrin homology; PKB, protein kinase B; NSCLC, non-small cell lung carcinoma; GSK-3, glycogen synthase kinase-3; CMV, cytomegalovirus; EGF, epidermal growth factor; EGFR, EGF receptor; ca-Akt, constitutively active Akt.
the PI3K-Akt cascade, PI3K phosphorylates lipids to form second messengers P(3,4,5)P3 and P(3,4)P2 in response to extracellular stimuli. The products of PI3K bind the PH domain and cause the translocation of Akt (also termed PKB) to the plasma membrane. At the plasma membrane, Akt is phosphorylated at Thr-308 by 3’-phosphoinositide-dependent kinase 1, whereas 3’-phosphoinositide-dependent kinase 2 has been suggested to phosphorylate Akt at Ser-473 (24, 25). Akt releases from the plasma membrane and inactivates proapoptotic molecules BAD, pro-caspase-9, and the Forkhead transcription factor (26).

When PI3K-Akt is not activated, the cellular antiapoptotic signals are attenuated, allowing the release of proapoptotic elements like BAD, pro-caspase-9, and the Forkhead transcription factor, thereby promoting cell death. This is followed by an increase in the expression of pro-apoptotic proteins such as Bax and caspase-9, leading to the activation of caspase-3 and the induction of apoptosis.

**Materials and Methods**

**Cell Culture and Transient Transfections.** The human NSCLC cell line H157 and the breast carcinoma cell line MCF7 were cultured at 37°C in a humidified chamber of 5% CO2 in RPMI 1640 and DMEM medium, respectively, supplemented with 10% FBS, 100 units/ml penicillin, and 100 µg/ml streptomycin. For DNA fragmentation, ELISA experiments with transiently transfected cells, cells (5 x 103/well) were grown in 96-well plates and treated, in triplicates, for 24 h with the indicated doses of paclitaxel, U0126, and LY294002. After treatment, the 96-well plates were centrifuged (200 x g) for 10 min. The supernatant was discarded, lysis buffer was added, and samples were incubated at room temperature following the manufacturer’s instructions. Anti-histone biotin and anti-DNA peroxidase antibodies were added to each well and incubated at room temperature for 2 h. After three washes, the peroxidase substrate was added to each well, and the plates were read at 405 nm after a 15-min incubation. The enrichment of histone-DNA fragments treated cells is expressed as fold increase in absorbance as compared with control (DMSO-treated) cells.

**Immunoprecipitation and Immunoblot Analysis.** Cells were serum starved for 16 h and lysed in 20 mM HEPES (pH 7.3), 50 mM sodium fluoride, 10% glycerol, 1% Triton X-100, 5 mM EDTA, and 0.5 mM NaCl supplemented with the tyrosine phosphatase inhibitor sodium orthovanadate (1 mM) and the protease inhibitors aprotinin (6 µg/ml) and leupeptin (10 µg/ml). Nuclei and insoluble material were removed by centrifugation at 13,000 x g for 10 min at 4°C. Receptor proteins were precipitated with various antibodies: HER2, clone 9G6.10 mouse monoclonal antibody (Neomarkers, Inc.); HER3 and HER4, polyclonal rabbit antisera raised against recombinant glutathione-S-transferase fusion proteins of HER3 and HER4, respectively, and protein A/G agarose beads (Santa Cruz Biotechnology) for 3 h at 4°C. Immune complexes were washed three times with lysis buffer, and protein samples were separated on an 8% SDS-polyacrylamide gel. The proteins were transferred to a polyvinylidene difluoride membrane and probed overnight at 4°C with anti-phosphotyrosine antibody PY20 (Santa Cruz Biotechnology). For immunoblot analysis, cells were lysed in 1% PBS, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na3VO4, 10 µM leupeptin, and 10 µM pepstatin at 4°C. Cellular proteins were quantitated by the Bradford assay, and equivalent amounts of proteins were resolved by 12% SDS-polyacrylamide gels. The proteins were transferred to nitrocellulose membranes and probed with anti-ERK monoclonal antibody for phosphorylated ERK1/2 (Santa Cruz Biotechnology), anti-ERK1/2 antibody (Santa Cruz Biotechnology), or anti-Akt (New England Biolabs) antibody to Akt1, Akt2, and Akt3. The secondary antibodies were conjugated with horseradish peroxidase, and protein levels were detected by enhanced chemiluminescence (Pierce).

**Akt Kinase Assay.** Cells were treated concurrently with the indicated concentrations of paclitaxel with or without 10 µM LY294002 (4, 10, and 50 µM) for an additional 24 h. Floating and adherent cells were collected and stained with 0.4% of trypan blue for 5 min at room temperature before being examined under the microscope. The numbers of viable cells were determined by trypan blue exclusion, and the results are expressed as the absolute numbers of viable cells. The floating and adherent dead cells that stained blue were scored positive and counted against the total number of cells to determine the percentage of cell death.

**DNA Fragmentation ELISA.** Quantitation of apoptotic cell death was determined by Cell Death ELISA (Roche Biochemicals) that measures cytoplasmic histone-DNA fragments produced during apoptosis. Briefly, cells (5 x 104/well) were grown in 96-well plates and treated, in triplicates, for 24 h with the indicated doses of paclitaxel, U0126, and LY294002. After treatment, the 96-well plates were centrifuged (200 x g) for 10 min. The supernatant was discarded, lysis buffer was added, and samples were incubated at room temperature following the manufacturer’s instructions. Anti-histone biotin and anti-DNA peroxidase antibodies were added to each well and incubated at room temperature for 2 h. After three washes, the peroxidase substrate was added to each well, and the plates were read at 405 nm after a 15-min incubation. The enrichment of histone-DNA fragments treated cells is expressed as fold increase in absorbance as compared with control (DMSO-treated) cells.
U0126. Akt kinase activity was measured according to the manufacturer’s (Roche Biochemicals) instructions. Briefly, endogenous Akt was immunoprecipitated from the cell lysates and incubated with the GSK-3 fusion protein, 200 μM cold ATP, and kinase buffer. GSK-3 was phosphorylated by Akt, and GSK-3 phosphorylation was measured by Western blotting using a phospho-GSK-3 antibody (1:1000). The result was quantified by pixel intensity with ImageQuant software (Molecular Dynamics).

Northern Blot Analysis. Northern analysis was performed using 100 ng of mRNA. The mRNA was isolated using Oligotex direct purification (Qiagen), electrophoresed on formaldehyde gels, and blotted onto nylon membranes (34). Probes were prepared by random priming of an isolated PCR amplified gene fragment (Prime-it II; Stratagene) for Akt2.

PI3K Lipid Kinase Assay. Cells were lysed and incubated with p85 antibody (Upstate Biotechnology) for 2 h at 4°C. The beads were washed with wash buffer A (1× PBS, 1% NP40, and 100 μM sodium vanadate), wash buffer B [100 mM Tris-HCl (pH 7.5), 500 mM LiCl, and 100 μM sodium vanadate], and wash buffer C [100 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, and 100 μM sodium vanadate]. The kinase reaction was initiated by the addition of 20 μg of phosphatidylinositol and 10 μl of 440 μM ATP, 20 mM MgCl2, and 30 μCi of [γ-32P]ATP. The samples were incubated for 10 min at 22°C with gentle agitation, and the reactions were terminated by the addition of 8 N HCl. The samples were then extracted with 160 μl of chloroform:methanol (1:1). The organic phase was concentrated by evaporation, and lipids were resolved by oxalate-treated thin-layer chromatography plates in chloroform:methanol:water:ammonium hydroxide (60:47:11.3:2). The phosphorylation products were visualized by autoradiography and quantified with a Storm PhosphoImager system (Molecular Dynamics) and ImageQuant software.

Results

Paclitaxel and MEK Inhibitor Induce Apoptosis in Human Lung Cancer H157 Cells. In a previous report, we established that low-dose paclitaxel activates endogenous ERK1/2, and MEK inhibition blocks paclitaxel-induced ERK1/2 activation (21). When used in combination, paclitaxel and MEK inhibition caused enhanced apoptosis, as demonstrated by the use of two different MEK inhibitors and a dominant-negative MEK in a variety of tumor lines. The enhanced apoptosis was verified by an ELISA for histone-associated DNA fragments, by terminal transferase-mediated dUTP nick end labeling assay, and by flow cytometric analysis for DNA content. Here, we confirm that concurrent treatment of paclitaxel (250 nM) plus U0126 (10 μM) enhances apoptosis using the ELISA that measures DNA-histone fragments (Fig. 1A).

To further confirm the relationship between apoptosis and cell death, we assessed total cell viability by trypan blue exclusion analysis. H157 cells were evaluated after concurrent treatment for 24 h with paclitaxel (250 nM) and U0126 (10 μM). H157 cells treated with a combination of paclitaxel and U0126 dramatically reduced viability compared with control cells. Twenty-seven % of the cells treated with a combination of paclitaxel and U0126 remained viable as determined by trypan blue exclusion. In contrast, 66% of the paclitaxel-treated cells and 90% of the U0126-treated cells remained viable (Fig. 1B).

Receptor Tyrosine Phosphorylation in Response to HB-EGF, Herregulin, and Paclitaxel Stimulation. Growth factor signal transduction can be initiated with the binding of a ligand, such as EGF or heregulin, to its cognate EGFR. Cells differ in their EGFR family member expression, and the overexpression of EGFR family members is known to affect endogenous levels of MEK/ERK and PI3K/Akt signaling (35). This led us to examine receptor tyrosine phosphorylation in response to HB-EGF or heregulin stimulation. H157 cells were serum starved for 16 h and treated with diluent control, 100 ng of HB-EGF, 10 ng of heregulin β1, or 250 nM paclitaxel, for 10 min. Endogenous EGF, HER2, HER3, or HER4 receptors were immunoprecipitated (IP) and immunoblotted (IB) with antiphosphotyrosine (anti-PY) antibody. The results for EGFR family phosphorylation are representative of at least two independent experiments.
In contrast, heregulin only minimally induced tyrosine phosphorylation of HER3, whereas HER2 and HER4 were not responsive to heregulin in H157 cells. Because our previous work has shown that low nanomolar doses paclitaxel activates endogenous ERK1 and ERK2 after 15 min of drug treatment (21), we wanted to determine whether this is linked to changes in activated EGFR, HER2, HER3, or HER4. Receptor tyrosine phosphorylation of EGF, HER2, HER3, or HER4 was not induced by paclitaxel (250 nM) treatment; thus, any signal transduction effects mediated by paclitaxel is not attributable to activation of these EGFR family members in H157 lung carcinoma cells.

**The Combination of Paclitaxel with MEK Inhibitor Decreases Akt Kinase Activity.** The serine/threonine protein kinase Akt is increasingly recognized as a key cellular signal that promotes cell proliferation and survival. To relate apoptosis with cell survival pathways, we considered the possibility that paclitaxel and U0126 may affect the antiapoptotic PI3K-Akt pathway. We found that Akt kinase activity is inactivated by a combination of paclitaxel and U0126 (Fig. 2A). H157 cells were treated with paclitaxel, U0126, or a combination of paclitaxel and U0126. Cell extracts were prepared and incubated with an immobilized Akt antibody to selectively immunoprecipitate Akt from the cell lysates. The resulting Akt immunoprecipitate was incubated with its substrate, GSK-3, in the presence of ATP. Akt activity as assessed by GSK-3 phosphorylation was not reduced by paclitaxel (250 nM) or U0126 (10 μM) alone. Importantly, treatment of these cells with a combination of paclitaxel (250 nM) and U0126 (10 μM) rapidly reduced the level of Akt activity by 64–78% as compared with control (DMSO-treated) cells. Consistent with the decrease in Akt kinase activity at 5 and 15 min, paclitaxel and U0126 decreased Akt kinase activity at 16 and 24 h. The decrease in Akt kinase activity by the combination of paclitaxel and U0126 was verified in MCF7 breast carcinoma cells (Fig. 3).

The decrease in Akt was not reflected at the protein level because Western blot analysis of Akt1, Akt2, and Akt3 protein showed no change after treatment (Fig. 2B). Akt2 overexpression has been shown in primary tumors; consequently, we tested the combined treatment of paclitaxel with MEK inhibition on Akt2 expression. Northern blot analysis confirmed that paclitaxel, U0126, or the two combined had no detectable effect on Akt mRNA expression over a time course of treatment (Fig. 2C). Thus, the effect was not attributable to decreased expression of Akt protein but was attributable to decreased activation of the enzyme.

**Akt Activation Reverses Tumor Apoptosis.** If decreased Akt is indeed involved in drug-induced apoptosis, a constitutively active form of Akt should reverse this apoptotic process (36–38). To directly address this issue, a ca-Akt, which
PI3K activity is crucial for the enhanced apoptosis observed with paclitaxel and U0126.

Signal transduction cascades involve a host of interacting components, and cross-talk between cascades is common and varies dramatically between cell types. Deregulation, mutation, or ablation of signaling cascades may effect signaling in other cascades. Recent studies show the cross-talk between the Akt and Raf (30, 31) and lead us to determine whether ca-Akt was altering the Raf-MEK-ERK pathway in H157 tumor cells. We transiently transfected pCMV control and ca-Akt and measured ERK activity by phosphorylation. The introduction of ca-Akt did not alter ERK activity in untreated cells, cells treated with paclitaxel, U0126, or a combination of these two drugs (Fig. 4C). This indicates that the effect of ca-Akt is not causing a change in the Raf-MEK-ERK pathway as measured by ERK1/2 activation.

The Combination of Paclitaxel and MEK Inhibitor Decreases PI3K Activity. The cell survival effect of Akt is mediated primarily by upstream PI3K activation; however, Akt also can be activated independent of PI3K by N-myristoylation, which leads to constitutive membrane recruitment and activation (39). The effect of paclitaxel and U0126 on PI3K was investigated to identify events upstream of Akt that may elucidate the Akt inactivation by this combination drug treatment. PI3K is a heterodimeric protein consisting of a p85 regulatory subunit and a p110 catalytic subunit. To assay for PI3K activity, H157 cells were transiently transfected with wild-type Akt (wt-Akt) or ca-Akt. After 24 h, cells were treated with paclitaxel and 10 μM U0126 for 24 h, and apoptosis was assayed by ELISA that measures DNA-histone fragments. The results shown represent at least three independent experiments; bars, SD. C, expression of ca-Akt does not alter ERK1/2 activity. H157 cells were serum starved for 16 h and treated with the indicated concentrations of paclitaxel ± U0126 for 15 min. Cell lysates were subjected to immunoblot analysis with anti-ERK antibody for phosphorylated ERK1/2.

PI3K Inhibitor LY29402 in Combination with Paclitaxel Enhances Apoptosis. PI3K lies upstream of Akt and induces Akt activation. The data in Fig. 5A show that PI3K activation is reduced by concurrent treatment with low-dose paclitaxel and U0126. To gather evidence that the enhanced apoptosis induced by the combination of paclitaxel and MEK inhibitor treatment was mediated by the PI3K-Akt pathway, the MEK inhibitor was substituted with LY29402. LY294002 is a specific inhibitor that has been widely used to study the role of PI3K in various biological responses. When the PI3K inhibitor was used in place of the MEK inhibitor, it also greatly enhanced the apoptotic effects of paclitaxel (Fig. 5B). In this treatment, we used the lowest clinically relevant concentration of paclitaxel (10 nM) that both blocks normal cell cycle progression at the G2-M phase of the cell cycle and induces Raf-1 and ERK1/2 activation (12, 21). The potential use of low-dose chemotherapy is important, because lower dosages are more attainable during cancer therapy and likely to cause less toxicity in patients. Increasing concentrations of LY294002 from suboptimal (4 μM) to complete inhibition (50 μM) of PI3K combined with low-dose paclitaxel (10 nM) resulted in a dose-dependent enhancement of

**Fig. 4** Role of Akt activation on tumor apoptosis. A, expression of a ca-Akt restored Akt activity. H157 cells were treated with 250 nM paclitaxel, 10 μM U0126, or a combination of paclitaxel plus U0126 for 15 min. In vitro Akt kinase activity toward GSK-3 was assessed as in the legend to Fig. 2A. B, activation of the Akt pathway reversed paclitaxel and U0126-induced apoptosis. H157 cells were transiently transfected with wild-type Akt (wt-Akt) or ca-Akt. After 24 h, cells were treated with paclitaxel and 10 μM U0126 for 24 h, and apoptosis was assayed by ELISA that measures DNA-histone fragments. The results shown represent at least three independent experiments; bars, SD. C, expression of ca-Akt does not alter ERK1/2 activity. H157 cells were serum starved for 16 h and treated with the indicated concentrations of paclitaxel ± U0126 for 15 min. Cell lysates were subjected to immunoblot analysis with anti-ERK antibody for phosphorylated ERK1/2.
tumor apoptosis. At the highest concentration of LY294002, paclitaxel-induced apoptosis was enhanced by >6-fold over paclitaxel alone, which is similar to that reported previously for U0126 or PD98059 (21). In contrast, the PI3K inhibitor alone or low-dose paclitaxel alone only exerted a modest effect on cell apoptosis.

To further establish the relationship between this combination drug treatment and cell death, we assessed cell viability by trypan blue exclusion analysis. H157 cells were evaluated after concurrent treatment for 24 h with paclitaxel (250 nM) and LY294002 (4, 10, and 50 μM). The left panel of Fig. 5C shows the total number of cells that remained viable as determined by exclusion of the trypan blue dye. Paclitaxel alone caused a drop in cell viability, which is further reduced by the addition of LY294002 in a dose-dependent manner. Fig. 5C, right panel, shows the percentage of cells that are dead, as determined by the cells that stained blue with the trypan blue dye. LY294002 or paclitaxel caused a slight increase in cell death, 7 and 15%, respectively. However, the two together resulted in a dramatic increase in cell death ranging from 56 to 77%, and this increase is dependent on the dosage of LY294002 used in the experiment.

Discussion

The natural product paclitaxel (Taxol) is considered one of the most effective chemotherapeutic agents in a number of clinical settings including significant efficacy against tumors that have been resistant to conventional chemotherapy. Compounds that selectively inhibit components of signal transduction pathways, such as inhibitors of EGFR, NF-κB, or MEK combined with paclitaxel, represent potentially powerful anticancer therapies. Recently, paclitaxel in combination with Herceptin, a humanized anti-HER2 monoclonal antibody, has shown efficacy in the treatment of metastatic breast cancer. Most relevant to this study, the efficacy of paclitaxel alone has also been demonstrated in the treatment of both small cell and NSCLCs. More extensive work has been reported for NSCLCs, where taxanes have shown a response rate of 20–30%, with 1 year survival of 40–53%, and median response duration of 7–11
months (40, 41). Improving the response rate in NSCLC and expanding the usefulness of paclitaxel in the treatment of resistant tumors with small molecule inhibitors that selectively target signal transduction pathways combined with conventional cancer chemotherapy are promising new therapeutic strategies.

Previous ideas of chemotherapy are largely based on the rationale that the administration of chemotherapeutic drugs results in the death of tumor cells by apoptosis. However, recent studies have revealed that many conventional chemotherapeutic agents also trigger pathways that have antiapoptotic effects, potentially limiting the effectiveness of the chemotherapy. Paclitaxel represents one such example in that it activates the MAPK signaling pathways, specifically the proapoptotic JNK pathway and the cell survival ERK1/2 pathway (21). Signaling by ERK1/2 has been implicated in both the development and progression of tumors (42). Our work stems from our original findings that a combination of two pharmacological agents, paclitaxel which alters microtubule polymerization and activates ERK1/2, and U0126 which inhibits MEK-ERK activity in the presence of paclitaxel. Importantly, the two drugs in combination result in an impressive enhancement of tumor cell killing in ovarian, breast, and lung carcinoma cell lines.

PI3K functions in multiple signal transduction pathways by interacting with oncogenes that leads to cellular transformation in ovarian, breast, and NSCLC (43). The amplification or up-regulation of PI3K-Akt signal transduction results in the development of cancer; thus, targeting and down-regulating PI3K or Akt activity is critical for cancer therapy (44–46). Importantly, our study provides strong evidence to support the conclusion that the enhanced apoptosis observed with a combination of paclitaxel and U0126 is associated with a reduction of the prosurvival kinase, Akt. We also show that expression of activated Akt is sufficient to confer a high degree of protection against drug-induced apoptosis in NSCLC, suggesting that tumors with unusually high Akt activity or mutated Akt may be able to overcome this therapeutic strategy. PI3K activity is also down-regulated by a combination of paclitaxel and MEK inhibition, indicating that PI3K is an important upstream kinase affected by these two drugs. Furthermore, a combination of paclitaxel and a PI3K inhibitor can reproduce the effect of paclitaxel and MEK inhibitor. These results point toward the following model for the role of paclitaxel and MEK inhibition in tumor cell apoptosis. Paclitaxel induces the activation of endogenous JNK and prevents microtubule depolymerization, both of which are important in promoting apoptosis (13–15). Paclitaxel also induces the stimulation of the MEK-ERK pathway, which may promote proliferation, growth, and survival. Alone, MEK inhibitors block tumor cell proliferation and survival by interfering with ERK1/2 activation (47). In combination, paclitaxel and MEK inhibition leads to enhanced tumor cell apoptosis, and an important component of this enhanced apoptosis is attributable to the inactivation of the PI3K-Akt pathway.

The elucidation of signal transduction pathways that control cell survival and death is actively revolutionizing cancer therapy, as evidenced by the recent promises obtained with STIS171, which targets the bcra-1 oncogene translocation product (48, 49). Novel combination therapies using conventional and new drugs that are directed at new targets constituting signaling molecules must take into consideration the mechanisms of action the combined drugs have against a tumor. Paclitaxel-containing treatments have been standard therapy for ovarian, breast, and more recently NSCLC. This report represents the first time where paclitaxel in combination treatment has been shown to alter PI3K/Akt activity. Most relevant to this study, the MEK inhibitor CI-1040 is being evaluated in ongoing trials as a single agent and possibly in combination therapy with paclitaxel (47). Thus, combining conventional paclitaxel chemotherapy and new anticancer drugs that block MEK or PI3K may provide a novel drug combination for the treatment of cancer.

This study raises some important considerations as to the balance of survival and apoptotic signals in chemotherapy-induced apoptosis. Understanding that paclitaxel and MEK inhibitors mediate their effects through the PI3K-Akt pathways is important in determining the most effective therapeutic combination. For example, if a tumor has low levels or lacks MEK/ERK activity, the application of MEK inhibitors may not reduce tumor growth; instead the use of PI3K inhibitors in place of the MEK inhibitor could be more effective. In contrast, aberrant Akt/PI3K control in tumors may also affect the decision-making process in selecting the appropriate chemotherapeutic combination. Thus, a combination of genomic and proteomic typing of tumors coupled with a molecular and biochemical markers to understand the effect of chemotherapeutic agents alone and in combination should revolutionize cancer treatment.

Acknowledgments

We thank Dr. Channing Der for helpful comments and discussions. Dr. Sergei Makarov kindly provided the Akt construct.

References


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