# Paclitaxel suppresses the viability of breast tumor MCF7 cells through the regulation of EF1α and FOXO3a by AMPK signaling

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**Abstract.** Paclitaxel (Taxol), a potent drug of natural origin isolated from the bark of the Pacific yew, is widely used for treating ovarian, lung and breast cancers. Currently, there is little information regarding the specific mechanism underlying the anticancer activity of paclitaxel. In the present study, we found that 5-amino-1-β-D-ribofuranosyl-imidazole-4-carboxamide (AICAR), a well-known activator of adenosine monophosphate (AMP)-activated protein kinase (AMPK), downregulated the protein and mRNA levels of elongation factor 1 α (EF1α) in breast cancer MCF7 cells. Paclitaxel increased the phosphorylation of AMPK and also downregulated the expression of EF1α in MCF7 cells. In addition, paclitaxel increased the expression, as well as the phosphorylation of forkhead box O3a (FOXO3a). Phosphorylation of FOXO3a was suppressed in the presence of compound C, a specific AMPK inhibitor, suggesting the involvement of AMPK in paclitaxel-induced FOXO3a phosphorylation. The induction and phosphorylation of FOXO3a by paclitaxel were not observed in EF1α and AMPK knockdown cells. Co-treatment with AICAR resulted in increased susceptibility of cancer cells to paclitaxel-induced suppression of their viability and further enhanced paclitaxel-induced FOXO3a phosphorylation. These results suggest that the antitumor effects of paclitaxel in breast cancer are mediated by activation of the AMPK/EF1α/FOXO3a signaling pathway.

## Introduction

Paclitaxel (Taxol) is a potent anticancer agent derived from the Pacific yew tree; it acts through the overstabilization of cellular microtubules. This natural product causes disruption of the mitotic machinery and inhibition of cell growth (1-3). Paclitaxel also exhibits anti-angiogenic properties, expanding the application of this family of drugs, known as taxanes, to various tumor types in breast (4), prostate (5), ovarian (6) and lung cancers (7). The effects of paclitaxel are believed to be mediated by the stabilization of microtubule polymerization, which leads to cell cycle arrest at the mitotic phase of the cell cycle, induction of detectable DNA fragmentation, and apoptosis. Taken together, these processes result in tumor regression (8). However, the use of paclitaxel is limited because the precise mechanisms underlying its antitumor effects are not completely elucidated.

Adenosine monophosphate (AMP)-activated protein kinase (AMPK) is a cellular fuel sensor that monitors the AMP/ATP ratio and maintains cellular homeostasis (9). Metabolic stresses, including hypoxia, exercise, and starvation, lead to the activation of AMPK (10,11). AMPK induces apoptosis in several cell types (12-15). These findings suggest that AMPK signaling is a potential therapeutic target in cancer. However, the molecular mechanisms underlying AMPK-dependent apoptosis of cancer cells remain unclear.

Elongation factor 1 α (EF1α) is a ubiquitously expressed protein that plays a key role in the elongation cycle during translation. EF1α is also involved in GTP-binding protein activity during signal transduction and oncogenesis (16-22). It is the most abundant protein in normal cells, accounting for 1-2% of the total protein content, and is highly expressed in the brain, heart and skeletal muscle, i.e., tissues consisting largely of long-lived, terminally differentiated cells (23-25). Regulation of EF1α levels may be important for normal cell function. Rapidly growing cells usually exhibit a large increase in EF1α mRNA levels (26), whereas overexpression of EF1α correlates with the emergence of metastases (27).

Forkhead box O3 (FOXO3; FKHRL1) is a member of the FOXO family of forkhead transcription factors that promote resistance to oxidative stress, tumor suppression and longevity (28-33). FOXO proteins primarily act as potent transcriptional activators by binding to the conserved consensus core recognition motif TTGTTTAC (34,35). By upregulating specific gene expression programs, FOXO transcription factors promote cell cycle arrest, repair of damaged DNA, de-toxification of reactive oxygen species, apoptosis and autophagy (36-45).

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*Abbreviations:* AMPK, adenosine monophosphate-activated protein kinase; FOXO3a, forkhead box O3a; EF1α, elongation factor 1 α; AICAR, 5-aminoimidazole-4-carboxy-amide-1-D-ribofuranoside

*Key words:* adenosine monophosphate-activated protein kinase, elongation factor 1 α, forkhead box O3a, Taxol

Based on earlier reports suggesting that AMPK mediates antitumor effects, considerable attention has been focused on the role of AMPK in tumor cells. In the present study, we investigated the effects of paclitaxel on the activity of AMPK in breast tumor MCF7 cells. We revealed that paclitaxel activated AMPK in MCF7 cells, whereas EF1α and forkhead box O3a (FOXO3a) affected AMPK-induced growth inhibition. Our results provide new therapeutic possibilities for the use of AMPK modulators as antitumor agents.

## Materials and methods

*Experimental agents*. EF1α, caspase-3, GAPDH, AMPKα2, and GAPDH antibodies were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA) and New England BioLabs, Inc. (Ipswich, MA, USA). Phospho-specific AMPK ($Thr^{172}$) and anti-AMPK antibodies were purchased from Upstate Biotechnology (Temecula, CA, USA). A FOXO3a/FKHRL1 ($Ser^{253}$) antibody was purchased from Sigma-Aldrich (St. Louis, MO, USA). A phospho-specific FOXO3a/FKHRL1 ($Ser^{253}$) antibody was purchased from Millipore Corp. (Billerica, MA, USA). An anti-$p21^{Waf1}$ antibody was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). HRP-conjugated secondary antibodies were obtained from the Kirkegaard and Perry Laboratory (Gaithersburg, MD, USA). Paclitaxel was purchased from Sigma-Aldrich. 5-Amino-1-β-D-ribofuranosyl-imidazole-4-carboxamide (AICAR) and 6-[4-(2-piperidin-1-yl-ethoxy)-phenyl)]-3-pyridin-4-yl-pyrrazolo[1,5-a]-pyrimidine (compound C) were obtained from Calbiochem (San Diego, CA, USA).

*Cell cultures*. The MCF7 cell line was grown in a 1:1 mixture of RPMI-1640 (Gibco, Auckland, New Zealand) containing 0.584 g/l of L-glutamate, 4.5 g/l of glucose, 100 g/ml of gentamicin, 2.5 g/l of sodium carbonate and 10% heat-inactivated fetal bovine serum (FBS).

*Immunoblot analysis*. Cells were grown in 6-well plates and serum-starved for 24 h prior to the treatment with indicated agents. The medium was aspirated and the cells were washed twice in ice-cold phosphate-buffered saline (PBS) and lysed in 100 μl of lysis buffer [50 mM Tris-Cl (pH 7.4), 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1 mM EDTA and 150 mM NaCl]. The lysed samples were briefly sonicated, heated for 5 min at 95˚C, and centrifuged for 5 min. The supernatants were electrophoresed on 8% SDS-PAGE gels and transferred to polyvinylidene difluoride membranes. The blots were incubated overnight at 4˚C with a primary antibody. They were then washed 6 times in Tris-buffered saline/0.1% Tween-20 and probed for 1 h with an HRP-conjugated secondary antibody at room temperature. The blots were visualized using an ECL detection system (Amersham Biosciences, Buckinghamshire, UK).

*3-(4,5-Dimethythiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay*. The MTT assay was performed as a crude measure of cell viability. MCF7 cells were seeded at a density of $5x10^4$/ml in 96-well plates and allowed to grow for 24 h. The growth medium was replaced with serum-free medium 24 h prior to treatment. Subsequently, MTT reagents (10 μl/well; 7.5 mg/ml in PBS) were added and the cultures were incubated for 30 min at 37˚C. The reaction was stopped by addition of acidified Triton buffer [0.1 M HCl in 10% (v/v) Triton X-100; 50 μl/well], and tetrazolium crystals were dissolved by mixing on a plate shaker at room temperature for 20 min. The samples were then measured on a plate reader (Bio-Rad 450; Bio-Rad Laboratories, Richmond, CA, USA) at a test wavelength of 595 nm and a reference wavelength of 650 nm. The results are representative of experiments repeated at least in triplicate.

*Silencing EF1α and AMPK*. MCF7 cells were seeded in 6-well plates and allowed to grow to 70% confluence for 24 h. Transient transfections were performed with the transfection reagent (Lipofectamine 2000; Invitrogen, Paisley, UK) as per the manufacturer's instructions. In brief, EF1α (NM_001013367; Dharmacon, Lafayette, CO, USA), AMPKα1, and non-targeted control siRNAs were designed. siRNA (5 μl) and transfection reagent (5 μl; Lipofectamine 2000) were each diluted in 95 μl of the reduced serum medium (Opti-MEM; Invitrogen) and then mixed. The mixtures incubated for 30 min at room temperature and then added in drops to each culture well containing 800 μl of the reduced serum medium (Opti-MEM; final siRNA concentration, 100 nM). The medium was replaced with a fresh medium at 4 h after transfection. The cells were cultivated for 24 h, lysed, and the expression of AMPKα1 protein was assayed by western blotting.

*Semi-quantitative RT-PCR*. First-strand cDNA synthesis was performed using 1 μg of total RNA isolated from frozen tissues at 55˚C for 20 min using a ThermoScript II One-Step RT-PCR kit (Invitrogen). Amplification of cDNA was performed in the same tube using the ABI GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Warrington, UK). The reverse transcriptase was inactivated by heating to 94˚C. The following PCR conditions were used: 27 cycles for 30 sec at 94˚C, 30 sec at 56˚C and 30 sec at 72˚C, followed by 7 min at 72˚C. The number of PCR cycles used was optimized to ensure amplification in the exponential phase. Samples (10 μl) of each RT-PCR product were analyzed by agarose gel electrophoresis. The bands were stained with ethidium bromide and visualized under ultraviolet light. Band intensity quantification was determined by a gel documentation system (Gene Genius; Syngene, UK). The following primers were used: EF1α sense, 5'-GATATGGTTCCTGGCAAGCCC-3' and antisense, 5'-CATTTAGCCTTGTGAGCTTTC-3'; GAPDH sense, 5'-ATTTGGTCGTATTGGGCGCCTGGTCACC-3' and antisense, 5'-GAAGATGGTGATGGGATTTC-3'.

*Data analysis*. The data are expressed as mean ± SEM Statistical analyses were conducted using SigmaStat (SPSS Inc., Chicago, IL, USA). Differences were considered significant at P-value <0.05.

## Results

*AICAR downregulates EF1α expression in MCF7 cells*. To ascertain whether the activity of AMPK affects EF1α expression, MCF7 cells were treated with AICAR, a pharmacologic activator of AMPK, and then analyzed by RT-PCR. We observed

that *EF1α* mRNA levels were decreased in a time-dependent manner following AICAR treatment (Fig. 1A). In addition, examination of EF1α protein levels in AICAR-treated MCF7 cells by western blotting revealed that EF1α expression was significantly decreased following incubation with AICAR compared with the expression under control conditions (Fig. 1B). Phosphorylation of AMPK was increased by AICAR treatment, confirming the appropriate conditions of our assay. Taken together, these results indicate that AMPK can suppress the expression of EF1α in MCF7 cells.

*Paclitaxel activates AMPK*. EF1α is known to be critical for breast tumor cell maintenance. Thus, we hypothesized that AMPK-mediated downregulation of EF1α has an anticancer effect. To test this hypothesis, we determined whether EF1α is involved in paclitaxel-stimulated breast cancer MCF7 cell apoptosis. The phosphorylation status of AMPK in paclitaxel-treated MCF7 cells was examined to determine whether the effects of paclitaxel are mediated by AMPK activation. We performed western blotting using a phosphorylation-specific ($Thr^{172}$) AMPK antibody and found that the level of AMPK phosphorylation in paclitaxel-treated MCF7 cells increased in a time- and dose-dependent manner compared the level in the controls (Fig. 2A and B). This observation provided further indirect evidence of increased AMPK phosphorylation following the treatment of MCF7 cells with paclitaxel.

*Paclitaxel downregulates EF1α and suppresses the viability of MCF7 cells*. EF1α not only functions as a translation factor but also binds to and severs microtubules (17,18). To better understand the role of EF1α in MCF7 cells, we determined EF1α levels in paclitaxel-treated MCF7 cells by western blotting. We found that EF1α expression was decreased in a dose-dependent manner by paclitaxel (Fig. 3A). Paclitaxel also downregulated the expression of EF1α in a time-dependent manner (Fig. 3B). Then, the effect of paclitaxel on cell viability was assessed. Treatment with paclitaxel resulted in a reduction of cell viability in a dose-dependent manner (Fig. 3C). Paclitaxel also activated caspase-3, a key mediator of apoptosis (Fig. 3D). These results suggest that paclitaxel suppresses MCF7 cell viability through the downregulation of EF1α expression.

*Paclitaxel induces FOXO3a expression and increases FOXO3a phosphorylation in an AMPK-dependent manner*. FOXO3a belongs to the forkhead family of transcription factors, which are characterized by a distinct forkhead DNA-binding domain. This protein functions as a trigger for apoptosis and is known to be a tumor suppressor. To evaluate the activation of FOXO3a by paclitaxel, we analyzed the changes in FOXO3a levels in paclitaxel-treated MCF7 cells. The expression of FOXO3a and $p21^{Waf1}$, its downstream effector molecule, was induced by paclitaxel treatment (Fig. 4A). A similar pattern of FOXO3a expression was also observed after AICAR treatment (Fig. 4B). Furthermore, FOXO3a phosphorylation was transiently increased by paclitaxel treatment (Fig. 4C). To determine whether AMPK activity was involved in the effect of paclitaxel, we investigated FOXO3a phosphorylation following treatment with 10 μM compound C, an AMPK inhibitor. Paclitaxel-induced phosphorylation of FOXO3a was inhibited in cells pretreated with compound C (Fig. 4D). These results indicate that FOXO3a is involved in the paclitaxel-stimulated signaling pathway in an AMPK-dependent manner.

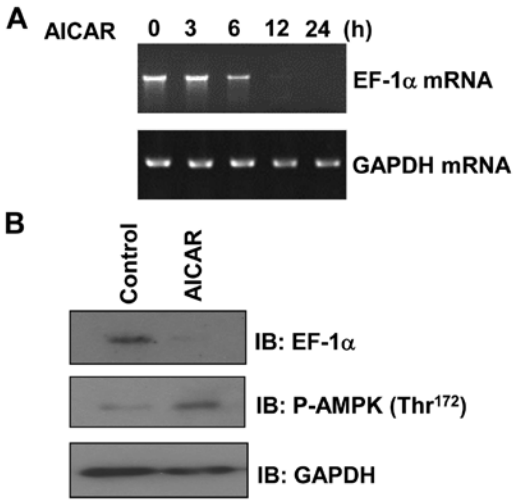


Figure 1. AICAR downregulates EF1α expression in MCF7 cells. (A) RT-PCR analysis of EF1α in AICAR-treated MCF7 cells. Total RNA was prepared from MCF7 cells after treatment with 1 mM AICAR. RT-PCR was performed using a specific EF1α primer. The PCR products were electrophoresed on 2% agarose gels and visualized under ultraviolet light. *GAPDH* mRNA was used as the positive control. (B) Downregulation of EF1α protein levels in AICAR-treated MCF7 cells. MCF7 cells were exposed to 1 mM AICAR for 24 h. The cell lysates were analyzed by western blotting with phosphorylation-specific antibodies against AMPK ($Thr^{172}$) as well as an anti-EF1α antibody. An anti-GAPDH antibody was used as the protein loading control.

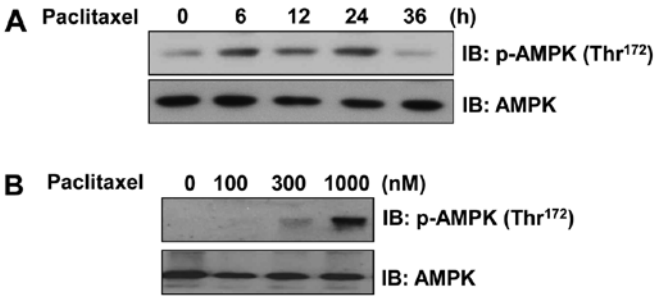


Figure 2. Paclitaxel activates AMPK. (A) Time-dependent phosphorylation of AMPK after paclitaxel treatment. Cells were exposed to 1 μM paclitaxel for different time periods. The cell lysates were analyzed by western blotting with phospho-specific AMPK ($Thr^{172}$) antibody. An anti-AMPK antibody was used as the protein loading control. (B) Dose-dependent phosphorylation of AMPK after paclitaxel treatment. Cells were treated with the indicated doses of paclitaxel for 24 h. The cell lysates were analyzed by western blotting with phospho-specific AMPK ($Thr^{172}$) antibody. An anti-AMPK antibody was used as the protein loading control.

*AMPK silencing blocks paclitaxel-induced EF1α downregulation and FOXO3a induction*. To explore the role of AMPK in the paclitaxel-activated biochemical pathway, we investigated the expression of EF1α and FOXO3 in AMPK-silenced cells. The expression of AMPKα1 was downregulated by AMPKα1 siRNA (Fig. 5A). The expression of EF1α and FOXO3a were suppressed in AMPK knockdown cells (Fig. 5B), suggesting the involvement of these molecules in AMPK-mediated

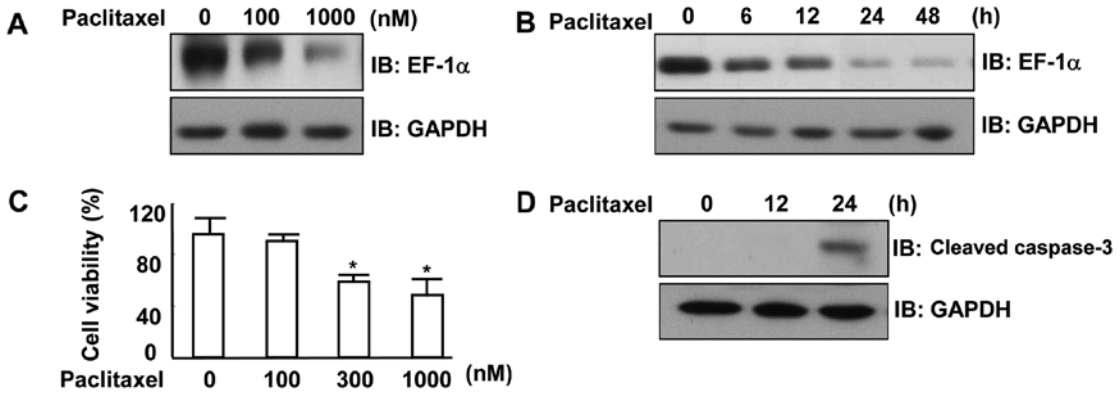


Figure 3. Paclitaxel downregulates EF1α and suppresses the viability of MCF7 cells. (A) Dose-dependent downregulation of EF1α expression by paclitaxel. Cells were exposed to the indicated doses of paclitaxel for 24 h. The cell lysates were analyzed by western blotting with an EF1α antibody. An anti-GAPDH antibody was used as the protein loading control. (B) Time-dependent suppression of EF1α in the course of paclitaxel treatment. Cells were treated with 1 $\mu$M paclitaxel for the indicated time periods. The cell lysates were analyzed by western blotting with an EF1α antibody. An anti-GAPDH antibody was used as the protein loading control. (C) Dose-dependent suppression of the viability of MCF7 cells after paclitaxel treatment. Cells were treated with the indicated doses of paclitaxel for 24 h. Values are expressed as the percentage of absorbance (MTT) relative to the control. All data are represented as the mean of experiments performed in triplicate. (D) Activation of caspase-3 by paclitaxel. Cells were stimulated with 1 $\mu$M paclitaxel for the indicated time periods. The cell lysates were analyzed by western blotting with an anti-cleaved caspase-3 antibody. An anti-GAPDH antibody was used as the protein loading control.

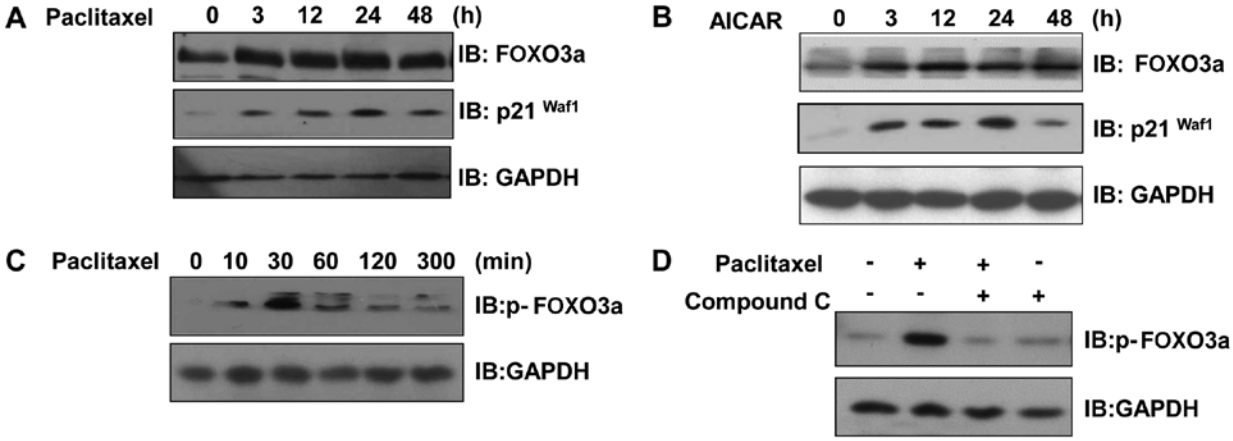


Figure 4. Paclitaxel induces FOXO3a expression and increases FOXO3a phosphorylation in an AMPK-dependent manner. (A) Time-dependent enhancement in FOXO3a and $p21^{Waf1}$ protein levels in response to paclitaxel treatment. Cells were exposed to 1 $\mu$M paclitaxel for the indicated time periods. Cells were analyzed by western blotting using antibodies to FOXO3a and $p21^{Waf1}$. An anti-GAPDH antibody was used as the protein loading control. (B) Time-dependent induction of FOXO3a and $p21^{Waf1}$ after AICAR treatment. Cells were exposed to 1 mM AICAR for the indicated time periods and analyzed by western blotting using antibodies against FOXO3a and $p21^{Waf1}$. An anti-GAPDH antibody was used as the protein loading control. (C) Time-dependent phosphorylation of FOXO3a by paclitaxel treatment. Cells were exposed to 1 $\mu$M paclitaxel for the indicated time periods and analyzed by western blotting using a phospho-specific FOXO3a antibody. An anti-GAPDH antibody was used as the protein loading control. (D) Paclitaxel potentiates FOXO3a phosphorylation in an AMPK-dependent manner. MCF7 cells were pretreated with compound C (10 $\mu$M) for 30 min and then stimulated with paclitaxel for 30 min. The cell lysates were analyzed by western blotting using an anti-phospho-FOXO3a antibody. An anti-GAPDH antibody was used as the protein loading control.

signaling. To confirm the role of AMPK in the regulation of FOXO3a and EF1α, we examined the effect of AMPK silencing on FOXO3a and EF1α levels in paclitaxel-treated cells. We found that paclitaxel failed to upregulate FOXO3a in AMPKα knockdown cells (Fig. 5B). Similarly, paclitaxel did not decrease EF1α levels in AMPKα knockdown cells. These results indicated that AMPK plays an important role in the regulation of EF1α and FOXO3a levels via paclitaxel-activated signaling.

*EF1α silencing potentiates the induction of FOXO3a by paclitaxel*. To confirm the role of EF1α in the regulation of cell viability and to exclude unrelated effects due to EF1α, we transfected MCF7 cells with double-stranded EF1α siRNA oligonucleotides. The cells were harvested 24 h after transfection, and EF1α expression was analyzed by western blotting. EF1α-specific siRNA completely suppressed EF1α expression, whereas scrambled siRNA (negative control) had no effect on EF1α levels (Fig. 6A). In addition, EF1α silencing increased the expression of caspase-3 and FOXO3a (Fig. 6A), key molecules for apoptosis, suggesting that EF1α may regulate apoptotic cell death. Moreover, knockdown of EF1α potently enhanced the induction of FOXO3a by paclitaxel (Fig. 6B). These results indicate that EF1α is essential for activation of the FOXO3a pathway by paclitaxel.

*AMPK activation sensitizes MCF7 cells to the suppression of their viability and induction of FOXO3a by paclitaxel*. To

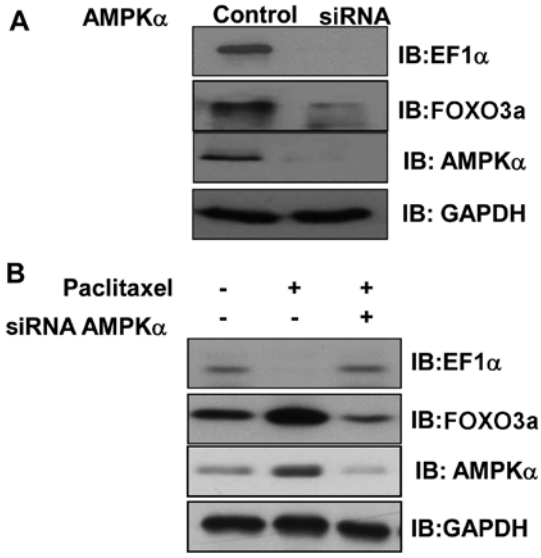


Figure 5. Silencing of AMPKα blocks paclitaxel-induced EF1α downregulation and FOXO3a induction. (A) MCF7 cells were transfected with AMPKα siRNA for 48 h. The cell lysates were analyzed by immunoblotting using antibodies against EF1α, FOXO3a and AMPKα. An anti-GAPDH antibody was used as the protein loading control. (B) Effect of AMPKα knockdown on paclitaxel-induced signaling. Cells were transiently transfected with AMPKα siRNA for 48 h and then treated with 1 $\mu$M paclitaxel for 24 h. The cell lysates were analyzed by western blotting using antibodies against EF1α, FOXO3a and AMPKα1. An anti-GAPDH antibody was used as the protein loading control.

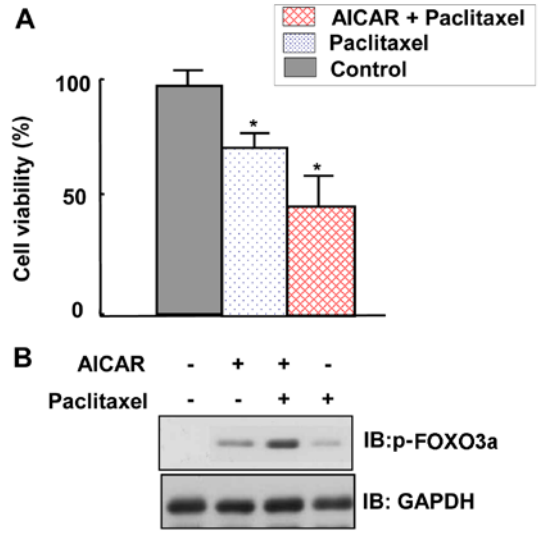


Figure 7. AMPK activation sensitizes MCF7 cells to paclitaxel-stimulated suppression of cell viability and FOXO3a induction. (A) Suppression of MCF7 cell viability following paclitaxel treatment in the presence or absence of AICAR. Cells were treated with 1 $\mu$M paclitaxel for 24 h with or without AICAR. Values are expressed as the percentage of absorbance measured by the MTT assay relative to the control. The data are represented as the mean of experiments performed in triplicate. (B) AICAR co-treatment sensitizes MCF7 to paclitaxel-activated FOXO3a signaling. MCF7 cells were treated with 1 $\mu$M paclitaxel for 30 min in the presence or absence of AICAR. The cell lysates were analyzed by immunoblotting using a phospho-specific FOXO3a antibody. An anti-GAPDH antibody was used as the protein loading control.

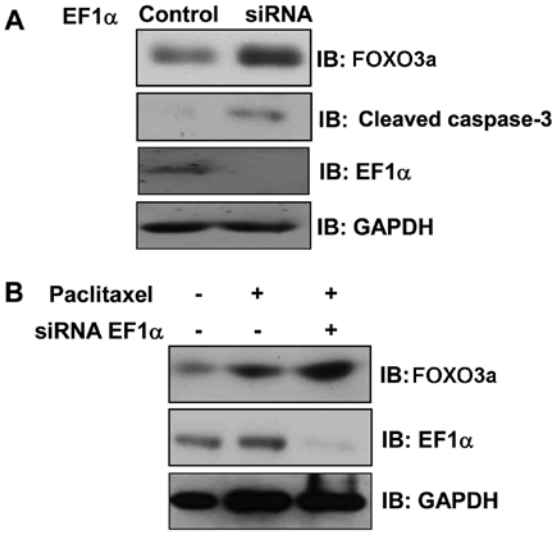


Figure 6. EF1α silencing potentiates FOXO3a induction by paclitaxel. (A) Effects of EF1α silencing on FOXO3a expression and caspase-3 activity. MCF7 cells were transfected with EF1α siRNA for 48 h. The cell lysates were analyzed by immunoblotting using antibodies against caspase-3, FOXO3a and EF1α. An anti-GAPDH antibody was used as the protein loading control. (B) EF1α-dependent FOXO3a activation. MCF7 cells were transfected with EF1α siRNA for 48 h and then treated with 1 $\mu$M paclitaxel for 24 h. The cell lysates were analyzed by immunoblotting using antibodies against FOXO3a and EF1α. An anti-GAPDH antibody was used as the protein loading control.

ascertain the role of AMPK in maintaining the viability of cancer cells, MTT assays were performed. The viability of MCF7 cells was inhibited by paclitaxel treatment (Fig. 7A). Moreover, co-treatment with AICAR further inhibited the viability of MCF7 cells (Fig. 7A). To gain insight into the mechanism by which paclitaxel suppresses MCF7 viability, FOXO3a expression levels were examined. We observed that FOXO3a expression was further increased by co-application of AICAR and paclitaxel compared with the expression following treatment with paclitaxel alone (Fig. 7B). In conclusion, these results indicate that AMPK activation increased the susceptibility of MCF7 cancer cells to the adverse effects of paclitaxel on cell viability.

## Discussion

In the present study, we demonstrated that paclitaxel activates AMPK and that this phenomenon may contribute to the suppression of breast tumor viability. The relationship between AMPK and paclitaxel has raised several questions regarding the mechanism by which paclitaxel can suppress tumor growth. The results of the present study suggest that the EF1α/FOXO3 pathway stimulated by paclitaxel may play a critical role in the suppression of tumor cells.

The aim of this study was to determine whether the activity of AMPK is directly regulated by paclitaxel, and if so, to uncover the molecules and signaling pathways involved in this regulation. The principal finding of this study is that EF1α is involved in the suppression of breast tumor MCF7 cell viability caused by paclitaxel. In addition, the results showed that EF1α is highly overexpressed in several tumor cell lines, including MCF7. The expression of EF1α is sensitive to the knockdown of AMPK and combined application of AICAR and paclitaxel. Considering our observations and the findings of previous studies that the AMPK pathway regulates transcription factors including EF1α (16,17), these results implicate the

interaction between AMPK and EF1α in the suppression of tumor viability by paclitaxel. EF1α is known to be associated with microtubules. Given that paclitaxel is a microtubule stabilizer, our results suggest that EF1α plays a critical role in the downstream cascades of AMPK by regulating cellular phenomena involving microtubules.

AMPK affects FOXO3a through a complex mechanism. FOXO3a is known to be a sensor of energy levels. AMPK, also a key cellular energy sensor, may phosphorylate FOXO3a in response to alterations in energy demands, thereby controlling protein stability. In the present study, we observed that paclitaxel increases the expression and phosphorylation of FOXO3a. Given that one of the AMPK phosphorylation sites on FOXO3a ($Thr^{179}$) is located in the DNA-binding domain, FOXO3a phosphorylation may affect the ability of this protein to bind DNA. Because the adenosine analog AICAR, an AMPK activator, augments FOXO3 levels, it is possible that AMPK may also transcriptionally regulate FOXO3.

We have demonstrated that paclitaxel treatment activates AMPK in breast tumor cells and that the EF1α/FOXO3 pathway is regulated by AMPK. These results suggest that the AMPK/EF1α/FOXO3 pathway may exert a profound influence on paclitaxel-induced inhibition of tumor viability. Future studies will be necessary to fully elucidate the role of AMPK in paclitaxel-activated signaling.

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