JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Demethoxycurcumin Modulates Prostate Cancer Cell Proliferation via AMPK-Induced Down-regulation of HSP70 and EGFR

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ABSTRACT: Curcumin (Cur), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) are major forms of curcuminoids found in the rhizomes of turmeric. This study examined the effects of three curcuminoid analogues on prostate cancer cells. The results revealed that DMC demonstrated the most efficient cytotoxic effects on prostate cancer PC3 cells. DMC activated AMPK and in turn decreased the activity and/or expression of lipogenic enzymes, such as fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC). AICAR, an AMPK activator, and DMC down-regulated heat shock protein (HSP) 70 and increased the activity of the pro-apoptotic effector, caspase-3. In addition, DMC sustained epidermal growth factor receptor (EGFR) activation by suppressing the phosphatases PP2a and SHP-2. DMC also increased the interaction between EGFR and Cbl and induced the tyrosine phosphorylation of Cbl. The results suggest that DMC may have antitumor effects on prostate cancer cells via AMPK-induced down-regulation of HSP70 and EGFR.

KEYWORDS: *curcuminoids, demethoxycurcumin, AMPK, EGFR, HSP70*

INTRODUCTION

Prostate cancer is a common urologic malignant tumor in men. It is the most common cancer, representing the second leading cause of cancer death.[1] Chemotherapeutic treatment options for castrate-resistant prostate cancer have a very modest palliative and survival benefit. To improve survival in prostate cancer, new therapeutic strategies to inhibit the appearance of this phenotype must be developed. Emerging studies on cancer prevention and treatment with natural products expand the traditional treatment of prostate cancer.

In contrast to normal human tissues, cancer cells display high rates of anabolic metabolism; overexpress lipogenic enzymes, including acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN); and show a high rate of energy consumption driving increased protein synthesis[2] and more active DNA synthesis.[3] Recent studies have shown that inactivation of lipogenic enzymes, such as FASN, ACC, and 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase), results in either cell death or growth inhibition in tumor cells.[4,5] Nearly all prostate cancer cells express high levels of FASN, suggesting that metabolic pathways have become an attractive target for drug discovery against prostate cancer.

AMP-activated protein kinase (AMPK), a highly conserved energy-sensing serine/threonine kinase, is activated by metabolic stress, for example, hypoxia, glucose deprivation, or exercise, which depletes intracellular ATP and increases AMP levels. Decreased AMPK activation is implicated in human metabolic disorders associated with increased cancer risk.[6] Interestingly, a recent study suggests that AMPK dysregulation may provide a mechanistic link between metabolic syndrome and prostate cancer.[7] Drugs that ameliorate metabolic syndrome conditions through AMPK activation may be beneficial for prostate cancer prevention and treatment.

A family of highly conserved chaperone proteins, heat shock proteins (HSPs), is dramatically increased in response to heat stresses. Previous studies have indicated that HSP70 promotes tumorigenesis via its prosurvival function.[8,9] High HSP70 expression, as observed in most cancer cells, has been associated with metastasis, poor prognosis, and resistance to chemotherapy or radiation therapy.[10] Furthermore, HSP70 antisense constructs selectively kill cancer cells, not only in cell culture but also in various orthotopic tumor xenografts in mice.[11−13] Thus, HSP70 seems to be an interesting molecular target for sensitizing tumor cells to cancer therapy. However, small molecules that are able to selectively inhibit HSP70 are still not known.

Deregulations of epidermal growth factor receptor (EGFR) signaling are often associated with abnormal cell growth and survival in prostate cancer cells.[14] Because ligand-dependent

Received: June 28, 2012
Revised: July 31, 2012
Accepted: July 31, 2012
Published: July 31, 2012

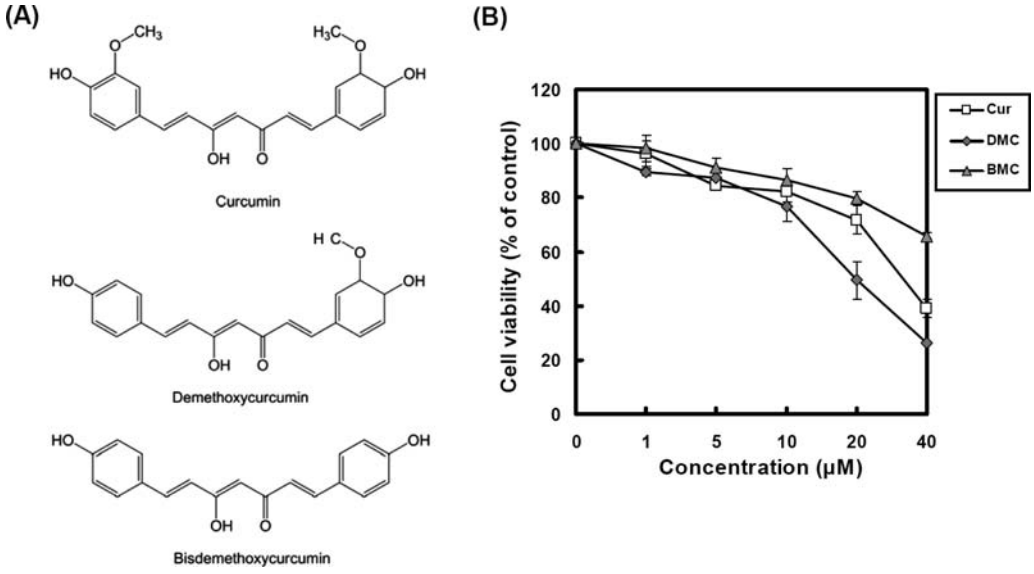

Figure 1. Proliferation-inhibitory effect of curcuminoids on prostate cancer cells: (A) structures of curcumin (Cur), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC); (B) PC3 cells treated with various concentrations of Cur, DMC, and BMC at 37 °C for 48 h. The effect on cell growth was examined by MTT assay, and the percentage of cell proliferation was calculated by defining the absorption of cells without pterostilbene as 100%. This experiment was repeated three times. Bar represent the SEM.

degradation of EGFR is a critical step for modulating receptor activity, targeting EGFR degradation may be an alternative approach to reduce cell signaling.[15] EGFR is monoubiquitinated at multiple sites through the action of the E3 ubiquitin ligases casitas B-lineage lymphoma (Cbl). Cbl has a tyrosine kinase binding (TKB) domain and a RING finger E3 ubiquitin ligase domain that are required for targeted protein degradation.[16] Cbl-mediated ubiquitination has been shown to play an important role during EGFR ubiquitination.[17] However, the relationship between AMPK and EGFR ubiquitination is less clear.

Curcuminoids are the major components that can be extracted from the rhizomes of *Curcuma longa* Linn.,[18] and they consist of a mixture of curcumin (Cur, 75−80%), demethoxycurcumin (DMC, 15−20%), and bisdemethoxycurcumin (BDMC, 3−5%). Recent studies have shown that curcuminoids possess a wide spectrum of physiological activity. They display anticancer activity,[19−21] promote neurite outgrowth,[22] possess antimutagenic properties,[23] and inibit influenza viruses.[24]

Currently, intense research efforts have focused on the potential of targeting metabolic pathways that may be altered during prostate tumorigenesis and prostate cancer progression. A significant amount of attention has been focused on the inhibition of tumor cell growth by the activation of AMPK. In the current study, we investigated the effects of curcuminoids on the viability of prostate cancer cells. Moreover, we plan to explore new therapeutic possibilities for the use of AMPK as an antiprostate tumor target.

MATERIALS AND METHODS

Materials. Compound **c** and antibodies for β-actin were purchased form Sigma (St. Louis, MO, USA). 5-Aminoimidazole-4-carboxamide-1-β-ribofuranoside (AICAR) was purchased from Toronto Research Chemicals (Toronto, ON, Canada). Antibodies for FASN, phospho-ACC (Ser 79), ACC, EGFR, phospho-EGFR, phospho-ERK1/2, ERK1/2, activated caspase-3, SHP-2, AMPK, and phospho-AMPK (Thr 172) were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibodies for HSP70 and tyrosine-specific antibody were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Cbl antibody was purchased from Upstate Technology (Lake Placid, NY, USA). Antibodies for mouse and rabbit conjugated with horseradish peroxidase (HRP) were purchased from Chemicon (Temecula, CA, USA). Western chemiluminescent HRP substrate was from Millipore Corp. (Billerica, MA, USA).

Drug Preparation. The powdered roots from *Curcuma longa* (2.5 kg) were extracted with ethanol (EtOH) at room temperature. The EtOH extract (0.57 kg) was removed in vacuo at 40 °C and triturated with *n*-hexane, chloroform ($CHCl_3$), and ethyl acetate (EtOAc) to give four fractions. The EtOAc-soluble fraction (20 g) was dissolved in methanol (MeOH) and then passed through a Sephadex LH-20 column, monitored by Si gel TLC analysis. A subfraction (2 g) was chromatographed on a Si gel column using a gradient elution (70 g, 70−230 mesh, 0−10% MeOH in $CHCl_3$) to give three pigments, Cur, DMC, and BDMC (>98% purity). Cur, DMC, and BDMC were dissolved in dimethyl sulfoxide (DMSO) and diluted in RPMI-1640 medium to different final concentrations in the following experiments.

Cell Culture. Human prostate cells and LNCaP, DU145, and PC3 cell lines were purchased from American Type Culture Collection. LNCaP, DU145, and PC3 cell lines were grown in RPMI-1640 media (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) (Invitrogen) and 1% penicillin−streptomycin (Invitrogen) at 37 °C in a humidified atmosphere of 5% CO_2.

MTT Assay. Cells (1×10^4) were seeded on the 24-well cell culture cluster overnight and then treated with different concentrations of agents as indicated in the figure captions and incubated for 48 h. Next, 40 μL of MTT (stock concentration = 2 mg/mL; Sigma Chemical Co.) was added to each well, and every volume of wells was 500 μL; this was followed by 2 h of incubation at 37 °C. MTT−formazan crystals will be formed; then 250 μL of DMSO was added to dissolve the crystals. Finally, an enzyme-linked immunosorbent assay (ELISA) reader was used to measure absorbance at OD 550 nm.

Western Blot Analysis. Cells (1×10^6) were seeded onto a 100 mm tissue culture dish containing 10% FBS RPMI-1640. Cells were then treated with various agents as indicated in the figure captions. After treatment, cells were placed on ice, washed with cold PBS, and lysed in lysis buffer. Western blot analysis was done as described previously.[25]

Immunoprecipitation. Cells were lysed with TGH buffer [1% Triton X-100, 10% glycerol, 50 mM HEPES (pH 7.3), 50 mM NaCl, 1 mM EGTA, 1 mM sodium orthovanadate, 10 mM sodium fluoride, 1

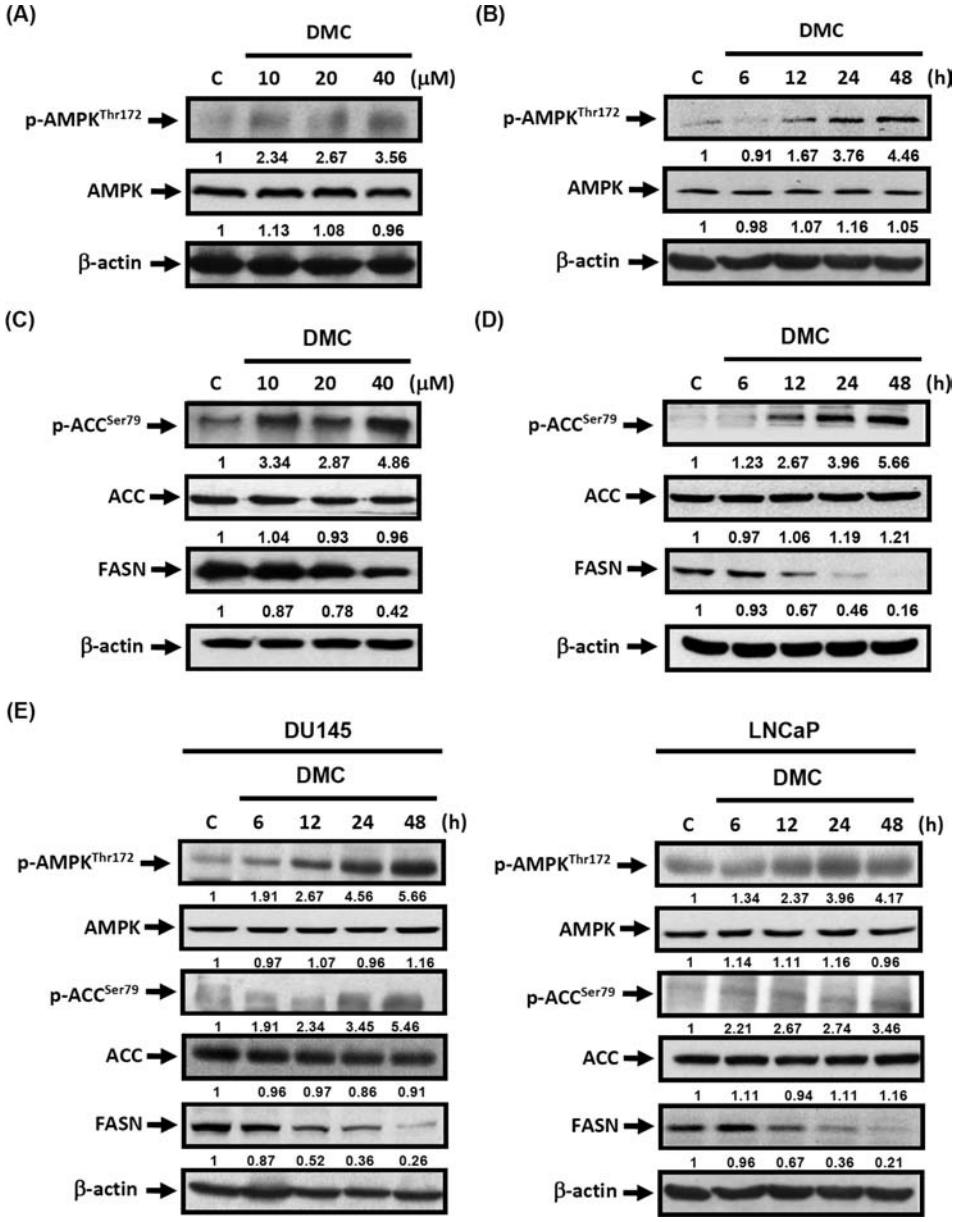

Figure 2. DMC activates AMPK and its downstream targets, ACC and FASN. (A) PC3 cells were stimulated with DMSO (control) or different concentrations of DMC for 48 h. Cell lysates were analyzed by Western blot with anti-phospho-AMPK and anti-AMPK antibodies. (B) PC3 cells were stimulated with DMSO (control) or 20 μM DMC for the indicated times. Cell lysates were analyzed by Western blot with anti-phospho-AMPK and anti-AMPK antibodies. (C) PC3 cells were stimulated with DMSO (control) or different concentrations of DMC for 48 h. Cell lysates were analyzed by Western blot with anti-phospho-ACC, anti-ACC, and anti-FASN antibodies. (D) PC3 cells were stimulated with DMSO (control) or 20 μM DMC for the indicated times. Cell lysates were analyzed by Western blot with anti-phospho-ACC, anti-ACC, and anti-FASN antibodies. (E) DU145 and LNCaP cells were stimulated with DMSO (control) or 20 μM DMC for the indicated times. Cell lysates were analyzed by Western blot with anti-phospho-AMPK, anti-AMPK, anti-phospho-ACC, anti-ACC, and anti-FASN antibodies. Western blot data presented are representative of those obtained in at least three separate experiments. Immunoblots were quantified, and relative expression to control is indicated.

mM phenylmethanesulfonyl fluoride, 10 mg/mL leupeptin, and 10 mg/mL aprotinin]. The lysates were then centrifuged at 14000*g* for 10 min at 4 °C. The supernatants were incubated with anti-EGFR and anti-Cbl antibodies for 4 h and then washed with TGH buffer three times. The immunoprecipitates were subjected to sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis.

Statistical Analysis. The results are expressed as the mean ± SEM. To analyze band intensity, Image Gauge (version 3.12, Fujifilm, Tokyo, Japan) was used. One-way ANOVA was used followed by Newman−Keuls multiple-range test to compare between groups. *P* values of <0.05 were considered to be significant.

RESULTS

Effect of Curcuminoids on Prostate Cancer Cells. The antitumor effect of curcuminoids has been successfully demonstrated in a wide range of human malignant cell lines.[19−21] To evaluate the preventive effects of curcuminoids against prostate cancer cells, the PC3 cells were treated with various concentrations of Cur, DMC, and BDMC (Figure 1A) for 48 h and examined for cell viability by MTT assay. As shown in Figure 1B, among these curcuminoids, DMC exhibited the most efficient cytotoxic effects on prostate cancer PC3 cells.

dx.doi.org/10.1021/jf302754w | J. Agric. Food Chem. 2012, 60, 8427−8434

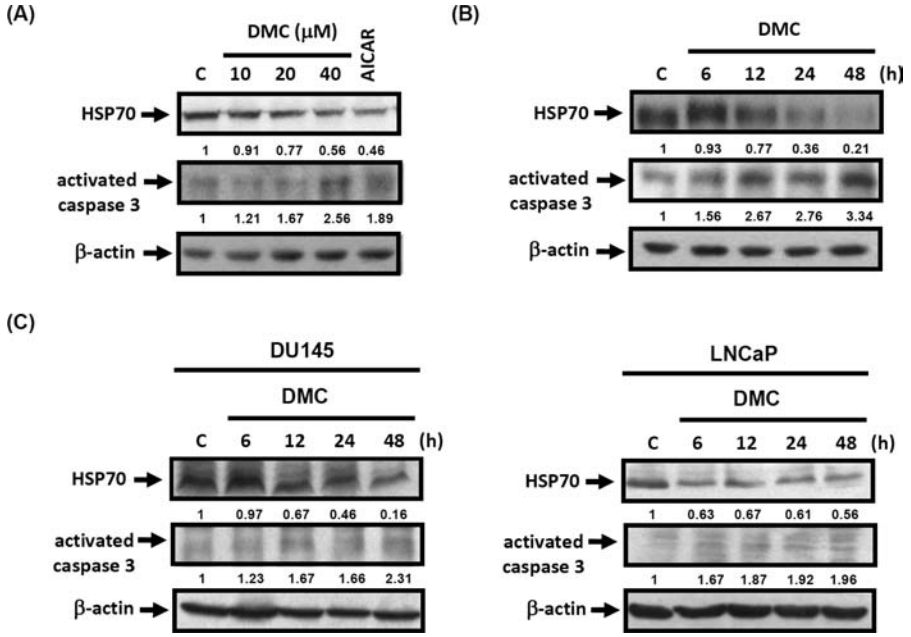

Figure 3. DMC down-regulates HSP70 expression in prostate cancer cells. (A) PC3 cells were stimulated with DMSO (control), 500 μM AICAR, or different concentrations of DMC for 48 h. Cell lysates were analyzed by Western blot with anti-HSP70 and antiactivated caspase-3 antibodies. (B) PC3 cells were stimulated with DMSO (control) or 20 μM DMC for the indicated times. Cell lysates were analyzed by Western blot with anti-HSP70 and antiactivated caspase-3 antibodies. (C) DU145 and LNCaP cells were stimulated with DMSO (control) or 20 μM DMC for the indicated times. Cell lysates were analyzed by Western blot with anti-HSP70 and antiactivated caspase-3 antibodies. Western blot data presented are representative of those obtained in at least three separate experiments. Immunoblots were quantified, and relative expression to control is indicated.

DMC Decreases Lipid Synthesis by Decreasing FASN Expression and Inhibiting ACC Activity via the Up-regulation of AMPK Activity. AMPK, as a tumor suppressor system, has gained more scientific interest because of its character as a negative regulator of tumor proliferation.[6] The level of AMPK phosphorylation in DMC-treated PC3 cells was examined to determine if DMC is involved in the regulation of AMPK. Using phosphorylation-specific antibodies for AMPK, we showed the phosphorylation of AMPK was increased in DMC-treated PC3 cells compared to control cells in a dose-dependent manner (Figure 2A). In addition, 20 μM DMC increased AMPK phosphorylation in a time-dependent fashion (Figure 2B). These results indicated that AMPK may mediate a DMC-associated signal pathway in PC3 cells. The activities of FASN and ACC were known to be negatively regulated by AMPK.[7] We next examined whether DMC decreases lipid synthesis by decreasing FASN expression and inhibiting ACC activity. PC3 cells were treated with different concentrations of DMC, and Western blot analysis indicated that DMC decreased the protein levels of FASN and increased ACC phosphorylation in a dose-dependent manner (Figure 2C). To address this time-dependent effect, we treated PC3 cells with 20 μM DMC for various periods of time. Western blot analysis indicated that DMC decreased the protein levels of FASN and increased ACC phosphorylation in a time-dependent manner (Figure 2D). To further confirm that the activation of AMPK by DMC is a general phenomenon, we treated DU145 and LNCaP cells with 20 μM DMC for various periods of time. Western blot analysis indicated that DMC increased AMPK phosphorylation, decreased the protein levels of FASN, and increased ACC phosphorylation in a time-dependent manner (Figure 2E). Our study established that the tumor prevention effect by natural products such as DMC may be attributed to AMPK activation.

DMC Down-regulates HSP70 Expression and Induces Caspase-3 Activation. The anti-apoptotic chaperone HSP70 is highly expressed in prostate cancer cells, which presents a major challenge for inducing apoptosis in this cell line.[10] Moreover, a recent study has identified the HSP70 pathway as being regulated by AMPK in HeLa cells.[26] To determine whether the activity of AMPK was correlated with HSP70 in prostate cancer cells, the HSP70 levels in AICAR-treated PC3 cells were analyzed by Western blotting. AICAR is a cell-permeable compound in which the phosphorylated metabolite activates AMPK. HSP70 expression was significantly decreased by treatment with AICAR (500 μM) in PC3 cells (Figure 3A). Caspase-3 activation also increased with the treatment of AICAR (Figure 3A). The effect of DMC on HSP70 expression was examined by Western blotting, which showed a dose- and time-dependent decrease in HSP70 levels (Figure 3A,B). DMC treatment also increased the activity of the pro-apoptotic effector, caspase-3, in a dose- and time-dependent manner (Figure 3A,B). To further confirm that the down-regulation of HSP70 by DMC is a general phenomenon, we treated DU145 and LNCaP cells with 20 μM DMC for various periods of time. Western blot analysis indicated that DMC decreased HSP70 expression and increased the activation of caspase-3 in a time-dependent manner (Figure 3C). Together, these results indicated that activation of AMPK suppresses HSP70 expression in prostate cancer cells.

AICAR Sensitizes PC3 Cells to DMC-Mediated Viability Suppression and Caspase-3 Activation. We next examined the chemosensitizing effect of AMPK on DMC in PC3 cells. Cells were treated for 48 h with DMC alone or in combination with AICAR, an AMPK activator. The fraction of cell growth inhibition clearly increased with combination DMC and AICAR treatment, compared to DMC or AICAR treatment alone (Figure 4A). To gain insight into the mechanism of

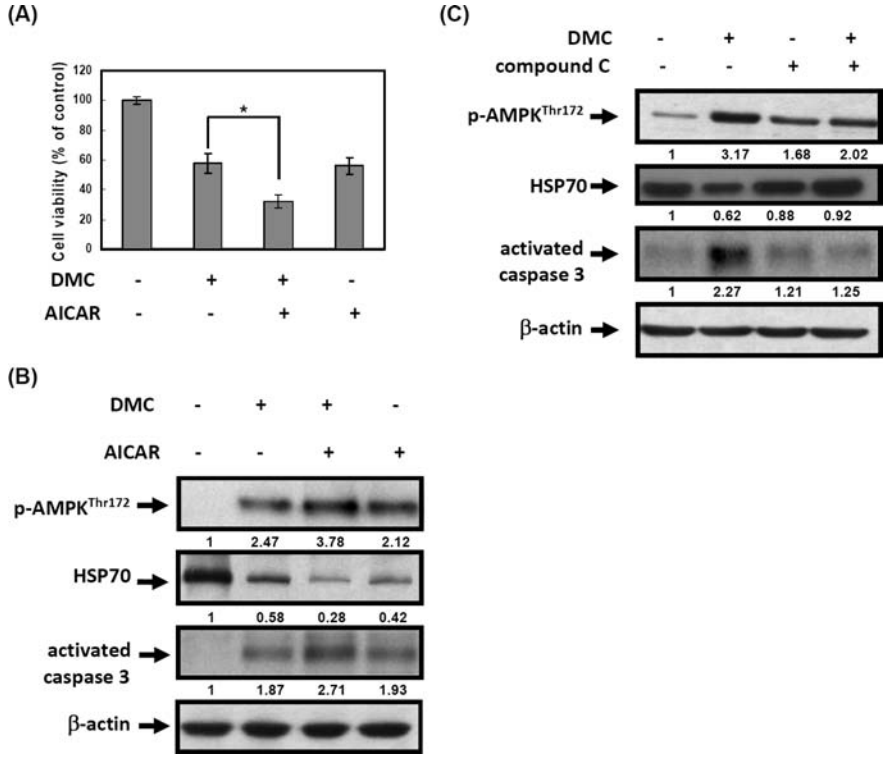

Figure 4. AICAR sensitizes PC3 cells to both DMC-mediated suppression of viability and activation of caspase-3. (A) Cells were treated for 48 h with either 20 μM DMC alone or cotreated with 500 μM AICAR. Values are expressed as the percentage of absorbance (MTT) relative to the control. Results are the means of triplicate experiments. (B) PC3 cells were treated for 48 h with either 20 μM DMC alone or cotreated with 500 μM AICAR. Cell lysates were analyzed by immunoblotting using antibodies specific for activated caspase-3, phospho-AMPK, and HSP70. An anti-β-actin antibody was used as the protein loading control. (C) PC3 cells were treated for 48 h with either 20 μM DMC alone or cotreated with 15 μM compound **c**. Cell lysates were analyzed by immunoblotting using antibodies specific for activated caspase-3, phospho-AMPK, and HSP70. An anti-β-actin antibody was used as the protein loading control. Western blot data presented are representative of those obtained in at least three separate experiments. Immunoblots were quantified, and relative expression to control is indicated.

AMPK, the effect of AICAR on DMC down-regulated HSP70 expression and induced caspase-3 activation was also determined. Down-regulation of HSP70 expression and activation of caspase-3 were potentiated by cotreatment of DMC with AICAR (Figure 4B). Phosphorylation of AMPK by combined DMC and AICAR treatment validated our experimental conditions (Figure 4B). To further study the effect of AMPK in regulating the HSP70 expression, compound **c**, an AMPK inhibitor, was added in the absence or presence of DMC. The expression of HSP70 was down-regulated, which confirmed the effect of DMC. The activity of caspase-3 was induced by the treatment of DMC, but there was no activation in the cotreatment of DMC with compound **c**. Moreover, down-regulation of HSP70 was blocked by cotreatment of DMC with compound **c** (Figure 4C), suggesting that AMPK is involved in DMC-mediated HSP70 down-regulation and caspase-3 activation. Taken together, these results indicate that AMPK sensitized PC3 cells to the effects of DMC-induced inhibition on PC3 cell viability.

DMC Down-regulates EGFR Expression and Induces EGFR Phosphorylation. EGFR is known to be essential for the growth and maintenance of prostate cancer.[14] To determine if the expression of EGFR is regulated by AMPK, EGFR levels in DMC-treated PC3 cells were analyzed by Western blotting. EGFR expression was significantly decreased by treatment with DMC in a dose- and time-dependent manner (Figure 5A,B). The effect of DMC on EGFR phosphorylation was also examined by Western blotting, which showed a significant increase in EGFR phosphorylation levels in a dose- and time-dependent manner (Figure 5A,B). The phosphorylation of ERK1/2, downstream of EGFR activation, was also increased by DMC treatment in a dose- and time-dependent manner (Figure 5A,B). These results demonstrated that DMC, an AMPK activator, suppressed EGFR expression and increased EGFR phosphorylation in PC3 cells. To determine if the phosphorylation of EGFR was correlated with phosphatase activity, the expression of PP2a and SHP-2 was examined in DMC-treated cells for 48 h. The expression of these phosphatases was significantly decreased by treatment with DMC compared to the controls (Figure 5C). These results suggest that DMC up-regulated EGFR phosphorylation through phosphatase down-regulation.

DMC Induces Cbl−EGFR Interaction. Cbl-mediated ubiquitination has been shown to play an important role during EGFR degradation. It is also known that the phosphorylation of Cbl might affect the EGFR−Cbl interaction status. Tyrosine phosphorylation of Cbl was increased by treatment with DMC for 48 h (Figure 6A). We next examined the protein−protein interaction between EGFR and Cbl, a ubiquitination ligase to EGFR, which might be regulated by DMC. The effect of DMC on the EGFR−Cbl interaction was also examined by immunoprecipitation, which showed a DMC-dependent increase of this interaction (Figure 6B). Our results suggest that Cbl, an important ubiquitin ligase to EGFR, may be involved in DMC-induced EGFR down-regulation.

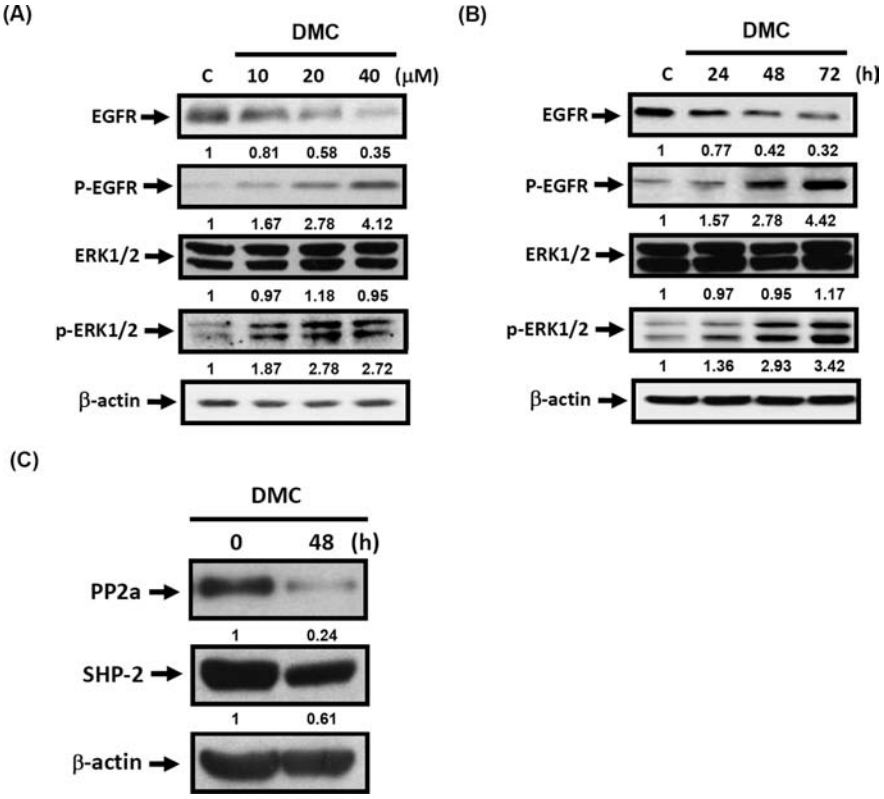

Figure 5. DMC down-regulates EGFR and activates EGFR phosphorylation. (A) PC3 cells were stimulated with DMSO (control) or different concentrations of DMC for 48 h. Cell lysates were analyzed by Western blot with anti-EGFR, anti-EGFR phosphorylation, anti-ERK1/2, and anti-ERK/12 phosphorylation antibodies. An anti-β-actin antibody was used as the protein loading control. (B) PC3 cells were stimulated with DMSO (control) or 20 μM DMC for the indicated times. Cell lysates were analyzed by Western blot with anti-EGFR, anti-EGFR phosphorylation, anti-ERK1/2, and anti-ERK/12 phosphorylation antibodies. An anti-β-actin antibody was used as the protein loading control. (C) PC3 cells were exposed to 20 μM DMC for 48 h. Cell lysates were analyzed by Western blot with anti-PP2a and SHP-2 antibodies. An anti-β-actin antibody was used as the protein loading control. Western blot data presented are representative of those obtained in at least three separate experiments. Immunoblots were quantified, and relative expression to control is indicated.

DISCUSSION

Resent studies have shown that Cur, a member of curcuminoids, has potent antitumor effects on prostate cancer.[27,28] However, Cur can be easily degraded both in vitro and in vivo.[29] The antitumor activity of Cur will be enormously reduced, and this instability of Cur limits its clinical use in cancer treatment. It is feasible to develop new stable compounds that are structurally similar to Cur but without the loss of antitumor activity. DMC, an analogue of Cur, is one such compound. In comparison with Cur, the structure of DMC lacks one methoxy group directly linking to the benzene ring (Figure 1A). Although the structure difference between Cur and DMC is slight, the chemical characteristic of DMC is more stable.[30] In the present study, we investigated the effects of three natural curcuminoids on proliferation of PC-3 cells. DMC was the most effective among three different curcuminoids. Several members of curcuminoids have potent antitumor effects. Simon et al. reported that DMC showed the highest antitumor bioactivity in three different curcuminoids to breast cancer cells.[31] DMC also induced G2/M phase arrest and apoptosis in human glioma U87 cells.[32] Consistent with these studies, we observed reduced proliferation in DMC-treated PC3 cells. Our results may provide a rationale for the potential use of DMC as a chemopreventive agent for prostate cancer.

The objective of our study was to determine if the viability of a tumor cell line was directly regulated by DMC and, if so, what molecules and signaling pathways were involved. We have demonstrated that DMC activates AMPK and that DMC contributes to the suppression of prostate cancer cell viability. Decreased AMPK activation is implicated in human metabolic disorders associated with increased cancer risk. Drugs that ameliorate metabolic syndrome conditions through AMPK activation may be beneficial for prostate cancer prevention and treatment. The association of AMPK with diminished tumor cell viability raises several questions regarding the mechanism by which DMC can suppress tumor growth. Pan et al. suggested that AMPK is a new molecular target of Cur and that AMPK activation partially contributes to the cytotoxic effect of Cur in ovarian cancer cells.[33] Interestingly, our study also found that DMC-induced AMPK activation plays a critical role in suppressing tumor cell growth.

De novo fatty acid and sterol syntheses are common features of prostate cancer. FASN and ACC are responsible for the synthesis of precursor of cholesterol, and their role in the pathogenesis and progression of prostate cancer is well established.[34] Series of studies showed that lipogenic enzymes are up-regulated or hyperactivated in cancer cells and that the inhibition of FASN or ACC limits cancer cell growth and survival.[35,36] Thus, DMC-induced ACC phosphorylation via AMPK activation may contribute to the cytotoxicity of DMC in prostate cancer cells.

dx.doi.org/10.1021/jf302754w | J. Agric. Food Chem. 2012, 60, 8427−8434

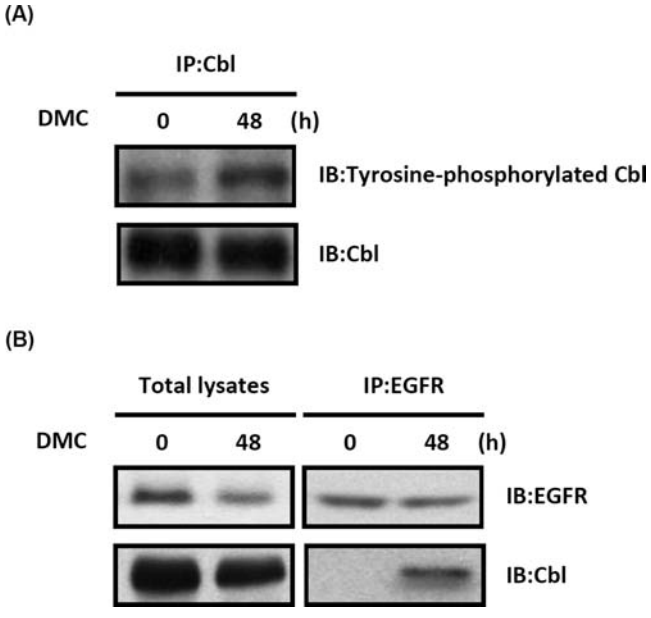

Figure 6. DMC induces Cbl−EGFR interaction. (A) PC3 cells were stimulated with 20 μM DMC for 48 h. Cell lysates were immunoprecipitated with anti-Cbl antibody. Immunoprecipitates were analyzed by Western blot with anti-tyrosine-specific and anti-Cbl antibodies. (B) PC3 cells were stimulated with 20 μM DMC for 48 h. Cell lysates were immunoprecipitated with anti-EGFR antibody. Immunoprecipitates were analyzed by Western blot with anti-Cbl or EGFR antibodies. Western blot data presented are representative of those obtained in at least three separate experiments.

Many studies have indicated that high levels of HSP70 expression are correlated with increased cell proliferation, lymph node metastasis, and poor survival.[10] The anti-apoptotic chaperone of HSP70 is thought to modulate signal transduction pathways and to have a role in proteasome-mediated degradation of apoptotic and survival proteins. HSP70 is highly expressed in PC3 cells, which presents a major challenge for inducing apoptosis in this cell line. Whether DMC exerts its pro-apoptotic affects through the direct down-regulation of apoptosis inhibitors or by inhibiting the chaperone function of HSP70 is unclear. In the present study, we hypothesized that DMC mediates the induction of cancer cell apoptosis through AMPK. We also hypothesized that AMPK is necessary for the down-regulation of HSP70. We cannot rule out the possibility of an indirect relationship between AMPK and HSP70 because we did not check the effect of DMC-mediated HSP70 regulation in AMPK dominant-negative cells.

The down-regulation of EGFR plays a fundamental role in the phenotypic changes of prostate cancer. We found that DMC decreases the expression of the phosphatases, PP2a and SHP-2, whereas phosphorylation of EGFR is elevated. Our study also found that treatment of PC3 cells with AICAR led to a modest decrease in the levels of expression of the phosphatases PP2a and SHP-2 (data not shown). These results indicate that AMPK induced EGFR degradation by regulating phosphatase expression in PC3 cells. Our results also find that Cbl, an important ubiquitin ligase to EGFR, may be involved in DMC-induced EGFR down-regulation. DMC increases the interaction between EGFR and Cbl and also induces the tyrosine phosphorylation of Cbl. The induction of the Cbl−EGFR interaction in DMC-treated cells, as shown in the current study, is likely due to some complex mechanism, which warrants further investigation.

In summary, we have demonstrated that DMC treatment activates AMPK in prostate cancer cells and that the HSP70 and EGFR pathways are regulated by AMPK. These results demonstrate that the AMPK pathway may exert a profound influence on DMC-mediated inhibition of tumor viability. Future studies will focus on elucidating the role of AMPK in DMC-mediated signaling.

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Funding

This study was supported by the National Science Council of the Republic of China Grant NSC 101-2320-B-039-031-MY3. We appreciate support (in part) by grants from the Department of Health (Taiwan), China Medical University Hospital Cancer Research Center of Excellence (DOH100-TD-C-111-005), and the China Medical University (CMU100-TS-10 and CMU99-COL-29-2).

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

ACC, acetyl-CoA carboxylase; AICAR, 5-aminoimidazole-4-carboxamide-1-β-ribofuranoside; AMPK, AMP-activated protein kinase; BDMC, bisdemethoxycurcumin; Cur, curcumin; DMEM, Dulbecco's modified Eagle's medium; DMC, demethoxycurcumin; DMSO, dimethyl sulfoxide; EGFR, epidermal growth factor receptor; FASN, fatty acid synthase; FBS, fetal bovine serum; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl CoA reductase; HSPs, heat shock proteins; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide.

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