AMP-activated protein kinase promotes human prostate cancer cell growth and survival

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Abstract
The molecular mechanisms underlying the development and progression of prostate cancer are poorly understood. AMP-activated protein kinase (AMPK) is a serine-threonine kinase that is activated in response to the hypoxic conditions found in human prostate cancers. In response to energy depletion, AMPK activation promotes metabolic changes to maintain cell proliferation and survival. Here, we report prevalent activation of AMPK in human prostate cancers and provide evidence that inhibition or depletion of AMPK leads to decreased cell proliferation and increased cell death. AMPK was highly activated in 40% of human prostate cancer specimens examined. Endogenous AMPK was active in both the androgen-sensitive LNCaP cells and the androgen-independent CWR22Rv1 human prostate cancer cells. Depletion of AMPK catalytic subunits by small interfering RNA or inhibition of AMPK activity with a small-molecule AMPK inhibitor (compound C) suppresses human prostate cancer cell proliferation. Apoptotic cell death was induced in LNCaP and CWR22Rv1 cells at compound C concentrations that inhibited AMPK activity. The evidence provided here is the first report that the activated AMPK pathway is involved in the growth and survival of human prostate cancer and offers novel potential targets for chemoprevention of human prostate cancer. [Mol Cancer Ther 2009;8(4):733–41]

Introduction
Prostate cancer is the most common nonskin malignancy and the second leading cause of cancer death in American men (1). It is believed that prostate cancer, like other malignancies, develops and progresses through an accumulation of genomic/proteomic alterations (2). Molecular studies have identified several candidate genes that are consistent with important aspects of biological features of prostate cancer and likely to be important in prostate cancer pathogenesis and progression (3). A number of these genes have important roles in regulating cellular metabolism, including androgen receptor (AR), phosphatase and tensin homologue deleted on chromosome 10 (PTEN), p53, and α-methylacyl-CoA racemase (AMACR), suggesting that metabolic changes may contribute to the development of prostate cancer.

The PTEN tumor suppressor gene is one of the most frequently deleted genes in prostate cancer. Loss of PTEN phosphatase causes the accumulation of phosphatidylinositol-3,4,5-triphosphate and the activation of downstream effectors such as Akt. Persistent Akt activation promotes metabolic changes that allow for prostate tumorigenesis, including up-regulation of biosynthetic pathways including glycolysis, protein synthesis, and fatty acid synthesis. Activation of Akt induces aerobic glycolysis (the Warburg effect) by affecting multiple proteins that are directly involved in glycolysis. In addition, activated Akt phosphorylates ATP citrate lyase (4), which cleaves citrate to form oxaloacetate and acetyl-CoA, promoting fatty acid synthesis. p53 can inhibit glycolysis by inhibition of phosphoglycerate mutase (5). Loss of p53 activity is required for Akt-dependent tumorigenesis (6). In addition, decreased expression of p53 targets, such as TP53-induced glycolysis and apoptosis regulator and synthesis of cytochrome oxidase 2, also drives glycolysis by decreasing the capacity of cells to use oxidative phosphorylation (7).

Hypoxia and metabolic stress are common characteristics of solid tumors (8). They result from an imbalance between tumor blood supply and tumor consumption. It has been suggested that hypoxia may lead to genetic instability, tumorigenesis, and disease progression (8). Similar to cervical and head and neck cancers, areas of relatively poor blood flow and hypoxia are found in localized prostate cancer (9–12). Immunohistochemical analysis of hypoxia markers (13, 14) and molecular imaging (15) support these findings. Importantly, tumor hypoxia has been...
shown to be an independent prognostic factor in prostate cancer progression (11, 16).

AMP-activated protein kinase (AMPK) is a serine-threonine kinase that is activated in response to the hypoxic conditions found in human prostate cancer. AMPK is a ubiquitous multisubunit serine-threonine protein kinase that forms heterotrimeric composed of a catalytic subunit (α1 or α2) and two regulatory subunits (β and γ; ref. 17). In response to metabolic stress, AMP levels are elevated and AMP binds to the γ subunit. AllostERIC changes promote phosphorylation of the activation loop of the catalytic subunit via upstream kinases (17–20). In response to energy depletion, AMPK activation promotes metabolic changes to maintain cell proliferation and survival by directly phosphorylating rate-limiting enzymes in metabolic pathways, modifying signal transduction cascades and gene expression (17, 21). AMPK has also been shown to stimulate glycolysis through direct phosphorylation and activation of 6-phosphofructo-2-kinase (PFK-2; refs. 17, 22). PFK-2 is the enzyme responsible for the synthesis of fructose-2,6-bisphosphate, a potent stimulator of glycolysis. In addition, AMPK activation mediates the recruitment of glucose transporters to the cell membrane (17, 23).

We hypothesized that AMPK is activated in prostate cancer and may act as a metabolic survival factor. AMPK activity in human prostate cancer samples was investigated by immunohistochemical analysis of the phosphorylation status of the AMPK substrate acetyl-CoA carboxylase (ACC; ref. 24). To specifically block AMPK activity in human prostate cancer cells, we developed small interfering RNAs (siRNA) that specifically target the catalytic subunits of AMPK. We show that AMPK is activated in human prostate cancer specimens and cell lines, and blocking AMPK activity slows proliferation and induces apoptosis of human prostate cancer cells.

Materials and Methods

Cell Culture and Reagents

RWPE-1 (American Type Culture Collection), a human prostate epithelial cell line immortalized by human papillomavirus 18, was cultured in keratinocyte serum-free medium supplemented with 50 μg/mL bovine pituitary extract and 5 ng/mL human recombinant epidermal growth factor (Invitrogen). CWR22Rv1, LNCaP cells, DU-145, and PC-3 cells (American Type Culture Collection) were cultured in RPMI 1640 (Mediatech) containing 10% fetal bovine serum, 2.5 mmol/L -glutamine, and penicillin-streptomycin (100 IU/mL and 100 μg/mL, respectively) at 37°C with 5% CO2. LNCaP cells were cultured in the presence of 0.5 nmol/L dihydrotestosterone (5-α-androstan-17β-ol-3-one; Sigma-Aldrich). Compound C (CC) and 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR) were obtained from Calbiochem.

Immunohistochemical Detection of Phospho-ACC in Human Prostate Specimens

Paraffin-embedded arrayed prostate cancer specimens (US Biomax, Inc.) containing normal (38) and malignant (244) prostate tissues were deparaffinized, rehydrated, boiled with citrate buffer (pH 6), treated with 0.3% H2O2 and preincubated in blocking solution (10% normal goat serum). The primary antibody, anti-phospho (p)-ACC (S79P; Cell Signaling Technology, Inc.), was incubated with the specimens at a concentration of 1:50 for 1 h at room temperature. Antigen-antibody complexes were detected using a horseradish peroxidase–complexed antirabbit secondary antibody (Dako Envision-Plus, Dako North America, Inc.). 3,3′-Diaminobenzidine (Dako) was used as chromogen, and hematoxylin as counterstain. For negative control, subtype-specific IgG was used. Human pancreas was used as a positive control tissue. Individual prostate sample scores were recorded in a blinded manner (S.P.C. and S.S.) using semiquantitative steps of increasing staining intensity, where 0 was undetectable, low immunostaining gave 1+, intermediate immunostaining gave 2+, and high immunostaining gave 3+ as a score. To discriminate among distributions, the Wilcoxon-Mann-Whitney test was done using StatXact software (Cytel).

Western Blot Analysis

Prostate cancer cells were lysed in the lysis buffer [10 mmol/L Tris-HCl (pH 7.6), 5 mmol/L EDTA, 50 mmol/L sodium chloride, 30 mmol/L sodium pyrophosphate, 50 mmol/L sodium fluoride, 1 mmol/L sodium orthovanadate, 1% Triton X-100, 1× protease inhibitor cocktail (Sigma-Aldrich)]. The protein samples were separated by SDS-PAGE and transferred onto Immun-Blot polyvinylidene difluoride membranes (Bio-Rad Laboratories). The membranes were blocked with 5% nonfat dry milk and probed with the following antibodies: anti-AMPKα, anti-p-AMPKα (T172P), anti-ACC, anti-p-ACC (S79P), anti-PARP-1 (Cell Signaling Technology), and anti-β-actin (Sigma-Aldrich). Chemiluminescent detection was done using enhanced chemiluminescence reagents according to the vendor’s instructions (Pierce).

Construction of Enhanced Green Fluorescent Protein-Transducer of Regulated CREB-Binding Protein-2 Expression Plasmid and Transfection

A cDNA clone encoding human transducer of regulated CREB-binding protein 2 (TORC2; GenBank accession no. BC05362) was obtained from Open Biosystems. For expression of enhanced green fluorescent protein (EGFP)-TORC2 fusion protein, the cDNA encoding TORC2 was cloned via PCR into pEGFP-N1 (Clontech). PCR fragments were generated using pairs of a forward primer, GAGGGACTCGAGGCCCACCATGGGACGTGGGGCCGGAC, and a reverse primer, TGGAGCAGATTCGTTGAACGTGCATCGTCCGAA, to introduce XhoI and EcoRI sites, respectively. After digestion with XhoI and EcoRI, the PCR-amplified fragment was cloned into pEGFP-N1. The cloned fragment was sequenced in its entirety to verify that no mutations were introduced via PCR. For transient expression of EGFP-TORC2, CWR22Rv1 and LNCaP cells were transfected with EGFP-TORC2 expression plasmid using FuGENE (Roche) as described by the manufacturer. A single immunoreactive band of appropriate molecular weight was identified on Western blots of EGFP-tagged TORC2-transfected cell extracts (data not shown).

**Fluorescence Microscopy**

CWR22Rv1 and LNCaP cells were grown in six-well tissue culture chambers to 50% confluency. Cells were transiently transfected with 1 μg of EGFP-TORC2 expression plasmid. After incubation for 24 h, transfected cells were treated with 10 μmol/L CC or 100 μmol/L AICAR for 2 h. Cells were washed twice with PBS and fluorescence microscopy was done. Images were obtained using DP Controller software (Olympus).

**SiRNA-Mediated Knockdown of AMPK Catalytic Subunits**

AMPKα1 (PRKAA1 On-TAplus SMART pool duplex, Dharmacon) and AMPKα2 (PRKAA2 Silencer Validated siRNA, Ambion) were used for AMPK knockdown. siRNAs were transfected into human prostate cancer cells using Lipofectamine RNAiMax following the manufacturer’s instructions (Invitrogen). Luciferase siRNA (Dharmacon) was used as a negative control.

**Measurement of Cell Number**

Cells were collected after trypsinization, resuspended in serum-containing medium, and counted. Cell number was determined using a Multisizer 3 Coulter Counter (Beckman Coulter).

**Bromodeoxyuridine Labeling**

Cells were treated with bromodeoxyuridine (BrdUrd) (10 μg/mL) for 10 min to allow BrdUrd incorporation into newly synthesized DNA and washed with PBS. They were then harvested after tryptophanization and fixed with ice-cold 70% ethanol. The fixed cells were incubated with 1% normal goat serum in PBS for 1 h, followed by staining with an Alexa Fluor 488–conjugated anti-BrdUrd antibody (Caltag Laboratories) and propidium iodide. The stained cells were analyzed using FACSort (Becton Dickinson).

**Cell Cycle Analysis**

Cells were harvested and fixed in 70% ethanol. The fixed cells were then stained with propidium iodide (50 μg/mL) after treatment with RNase (5 μg/mL). The stained cells were analyzed for DNA content using FACSort (Becton Dickinson). Cell cycle fractions were quantified with CellQuest (Becton Dickinson) or ModFit LT (Verity Software House).

**Results**

**AMPK Is Frequently Activated in Human Prostate Cancer**

We examined AMPK activity in normal and malignant human prostate tissues by immunohistochemistry. Because AMPK is the sole kinase that phosphorylates ACC on Ser79 (24), Ser79 phosphorylation was used as a measure of AMPK activation. The p-ACC (Ser79) antibody detects ACC (25) only when it is phosphorylated at Ser79. This antibody detected a single band of appropriate molecular weight on Western blot and its specificity was verified by phosphorylation inhibition studies. Immunostained sections were quantitated with a semiquantitative scoring method (0–3+) based on the intensity of staining. Normal prostate epithelia were either completely negative or showed only weak staining (intensity score of 0–1+) for p-ACC (see a representative sample in Fig. 1A). p-ACC was found only in the cytoplasm of primary human cancerous specimens (see a representative sample in Fig. 1B). Figure 1C gives the percent distribution of p-ACC staining (0–3) and tissue status (normal versus malignant). The distribution of p-ACC staining was statistically different between the normal and cancer tissues (P < 0.0001). Approximately 40% of the cancerous specimens exhibited medium to high staining (intensity score of 2–3+) for p-ACC. The prevalence and intensity of p-ACC staining did not correlate with the Gleason grade of the sample (data not shown). In summary, activated AMPK is expressed frequently in primary human prostate cancer specimens of various Gleason grades.

**AMPK Is Activated in Human Prostate Cancer Cell Lines**

We next examined AMPK activity in various human prostate cancer cell lines, including the androgen-sensitive LNCaP cells and the androgen-independent LNCaP C4-2B, CWR22Rv1, DU-145, and PC-3 cells. To evaluate AMPK activation, we assessed the phosphorylation of the AMPK activation loop (Thr172) and its direct substrate ACC. Western blot analyses reveal that high levels of p-ACC were found in all the examined cell lines (Fig. 1D). The phosphorylation levels of the AMPK catalytic loop were also highly elevated. We interpret these data as support for the hypothesis that AMPK is active in human prostate cancer cells.

AMPK activity is low in normal human cells grown under standard tissue culture conditions. Mammalian cells convert AICAR to 5-aminimidazole-4-carboxamide ribonucleoside, an AMP mimetic that is a known activator of AMPK (26, 27). To determine if AMPK activity could be induced in human prostate cells, nontumorigenic prostate epithelial cells (RWPE-1) and CWR22Rv1 cells were treated with or without AICAR (0.5 mmol/L) for various periods of time (Fig. 2A). Western blot analysis of untreated RWPE-1 cells revealed low basal levels of AMPK and ACC phosphorylation. AICAR induced the phosphorylation of AMPK and ACC in a time-dependent manner. In cancerous CWR22Rv1 cells, AICAR treatment did not induce a further increase in the phosphorylation of the AMPK activation loop. Only a minimal increase in the phosphorylation of Ser79 of ACC was observed in AICAR-treated cells. Increasing the length of treatment (Fig. 2A) or the AICAR concentration (data not shown) failed to increase the modest induction of ACC phosphorylation. Similar results were seen in the other human prostate cancer cell lines (data not shown). These results support the idea that AMPK is highly activated in human prostate cancer cells, and this activation is minimally enhanced by AMP.

Because AMPK activation has not been documented under nonstressed tissue culture conditions, we assessed the activation status of AMPK in vitro. TORC2 is phosphorylated by AMPK at Ser171 within an optimal consensus sequence for AMPK (28). Phosphorylation at this site by active AMPK restricts TORC2 to the cytoplasm. TORC2
was chosen as a good substrate for analyzing AMPK activity because Ser171 is phosphorylated specifically by AMPK family members (29, 30). To monitor TORC2 localization in vitro, we constructed a COOH-terminal EGFP-tagged TORC2 mammalian expression vector. As shown in Fig. 2B, TORC2 was localized in the cytoplasm and nucleus of subconfluent proliferating LNCaP and CWR22Rv1 cells. When these cells were treated with AICAR, there was a modest shift to the cytoplasm. Consistent with the constitutive activity of AMPK in prostate cancer cells, treatment of cells with CC, a small-molecule ATP competitive inhibitor of AMPK (31), promoted TORC2 nuclear translocation in both cell lines. Taken together, these data strongly supported the activated status of AMPK in human prostate cancer cells.

**Down-Regulation of AMPK Catalytic Subunits by SiRNA Inhibits Cell Proliferation in Human Prostate Cancer Cells**

To determine whether AMPK activity promotes growth in human prostate cancer cells, we developed an AMPK-siRNA that reduced the protein levels of AMPK catalytic subunits (α1 and α2) to <10% of control by 72 hours (Fig. 3A). Western blot analyses with the p-ACC antibody verified that treatment of cells with AMPK-siRNA inhibits AMPK activity in prostate cancer cell lines (Fig. 3A) as indicated by p-ACC level. Subconfluent cultures of human prostate cancer cells were transfected with siRNA targeting the AMPK catalytic subunits or control siRNA. As shown in Fig. 3B, silencing of the AMPK catalytic subunit genes caused a reduction in the number of cells whereas control cells continued proliferating normally (Fig. 3B). Similar results were obtained in the androgen-dependent LNCaP and androgen-independent CWR22Rv1 cells. Further support was provided by BrdUrd incorporation experiments, which revealed that AMPK-siRNA significantly decreased S-phase entry in both cell lines (data not shown).

**AMPK Inhibition by a Small Molecule Decreases Cell Proliferation and Promotes Apoptosis in Human Prostate Cancer Cells**

To support the findings obtained with siRNA, an alternative method of AMPK inhibition was used. CC was previously identified in a chemical library screen as a potent and specific ATP-mimetic competitive inhibitor of AMPK (31). Western blot experiments with a phospho-specific antibody to ACC verified that treatment of human prostate cancer cells with CC inhibits AMPK activity in a dose-dependent (Fig. 4A) and time-dependent manner (Fig. 4B). As expected, CC had no effect on the phosphorylation of the AMPK catalytic loop.

To explore the effect of CC on cell proliferation, cells collected at different time points after treatment with various concentrations of CC were counted. Treatment of CWR22Rv1 (Fig. 5A) or LNCaP cells (Fig. 5B) with CC resulted in a dose-dependent decrease in the total number of viable cells. The decrease in proliferation correlated closely with the level of AMPK inhibition (Figs. 4A and 5A and B). Dose-dependent inhibition of proliferation was confirmed.

**Figure 1.** AMPK is activated in human prostate cancer. p-ACC expression in normal human prostate (A) and primary human prostate cancers (B) was analyzed by immunohistochemical staining with a polyclonal anti–p-ACC antibody in paraffin-embedded tissue sections. C, staining intensity of p-ACC in normal and malignant prostate tissues. D, Western blot analyses of extracts from various human prostate cancer cell lines. Cell lysates were analyzed by immunoblotting with anti–p-AMPK, anti-AMPK, anti–p-ACC, or anti-ACC antibody. Blots were probed with anti-β-actin antibody to normalize for protein loading.
by BrdUrd incorporation studies (Fig. 5C). BrdUrd is incorporated as a thymidine analogue into newly synthesized DNA and allows assessment of the proportion of cells cycling through S phase. BrdUrd incorporation was reduced from ∼23% to ∼2% of cells with CC (10 μmol/L) treatment for 24 hours (Fig. 5C).

Microscopic assessment of the effect of CC on prostate cancer cell viability revealed morphologic changes consistent with apoptotic cell death, including cell rounding, detachment, shrinkage, and blebbing (data not shown). To test whether CC-treated cancer cells die through induction of apoptosis, PARP-1 cleavage was analyzed. Western blot
experiments with the anti–PARP-1 antibody verified that treatment with CC induced PARP-1 cleavage in a dose-dependent (Fig. 6A) and time-dependent manner (Fig. 6B). The concentration of CC required for cell killing corresponded closely with the dose of CC required for AMPK inhibition (Figs. 4A and 6A). AICAR treatment with concentrations that activate AMPK (32) did not promote PARP-1 cleavage (Fig. 6A and B). Apoptotic cell death of prostate cancer cells treated with a concentration of CC that inhibits AMPK was also verified by cell cycle analysis. As shown in Fig. 6C, extensive DNA fragmentation (hypodiploid cell fragments) was detected in CC-treated cells. The percentages of cellular DNA in sub-G1 were ~2% in control cells and ~38% in CC-treated cells.

Discussion

Under stressed conditions, such as those found in prostate cancer, activated AMPK plays a major role in maintaining energy homeostasis by inducing ATP-producing catabolic pathways (33). In this study, we compared the levels of AMPK activity in normal and malignant prostate specimens by examining the phosphorylation status of the well-characterized AMPK substrate ACC. As expected, low levels of p-ACC were detected in nonstressed normal human prostate tissues. To our knowledge, this is the first report that shows prevalent AMPK activity in human prostate cancer specimens. This finding suggests that prostate cancer cells are energetically stressed due to their environment and the demands of continuous cell proliferation. The degree of AMPK activity varied among the cancerous specimens. This suggests that human prostate cancers vary in their levels of metabolic stress. Of note, we did not see a correlation between AMPK activity and tumor Gleason grade. This finding is consistent with microelectrode studies reported by others, showing no relationship between pO2 levels and Gleason grade (10). Alternatively, low AMPK activity in some of the cancerous specimens could be secondary to deficiencies in upstream kinases as seen in other cancer types (34). The selective activation of AMPK in prostate cancer specimens raises the question of a possible connection between AMPK activation and prognosis, which is currently being explored in our laboratory.

Surprisingly, we found that AMPK was highly activated not only in human prostate cancer specimens but also in human prostate cancer cells growing under standard tissue culture conditions. We hypothesize that AMPK activation is an important downstream effector of an unknown genomic and/or proteomic change found in transformed prostate epithelial cells (2). Transformed cells show altered metabolism when compared with normal cells. One of the most fundamental metabolic alterations occurring with malignant transformation is the up-regulation of aerobic glycolysis, a phenomenon known as the Warburg effect (35, 36). Currently, the molecular mechanisms leading to constitutive up-regulation of aerobic glycolysis are poorly understood (37). Activated AMPK, a possible contributor to the Warburg effect, has been shown to promote glycolysis by enhancing glucose uptake (17, 22) and activating PFK-2 (17, 23). In addition, AMPK is known to induce numerous glycolytic genes (38). Further studies are required to determine the relationship between transformation, aerobic glycolysis, and AMPK activity in human prostate cancer.

The mechanism by which AMPK is activated in human prostate cancer cells is currently unclear. Under normal physiologic conditions, AMPK is activated under conditions that deplete cellular ATP, such as glucose deprivation, heat shock, hypoxia, and ischemia (17). However, AMPK activity may also be elevated under nonstressed conditions. For example, AMPK is activated by hormones like leptin, adiponectin, and interleukin-6 (39). These adipokines have been implicated in the development and progression of human prostate cancer (40). LKB1 and CAMKKβ are central candidates for AMPK activation in prostate cancer because these enzymes are responsible for AMPK activation in noncancerous tissues (17–20). Whereas LKB1 is a known tumor suppressor (41), CAMKKβ activation has not been linked to prostate cancer progression. Alternatively, an unidentified kinase(s) could be acting as an AMP-activated kinase kinase in prostate cancer.

In normal cell physiology, AMPK activation has been proposed to protect cells from injury due to hypoxia and other metabolic stressors (42) by slowing cell growth and proliferation. The target of rapamycin (TOR) stimulates the initiation step of protein synthesis, which is required for cell growth via phosphorylation of multiple targets (43). TOR is activated by Akt phosphorylation of its binding

Figure 4. Dose-dependent and time-dependent inhibition of AMPK activity by CC. CWR22Rv1 cells were treated with various concentrations of CC for 24 h (A) or with CC (10 μmol/L) for various times as indicated (B). Samples were collected and analyzed by Western blots with antibodies for p-ACC, ACC, phospho-AMPK, and AMPK. Blots were probed with anti-β-actin antibody to normalize for protein loading.
partner Raptor and an upstream pathway involving tuberous sclerosis complex 2 (TSC2; ref. 44). AMPK inhibits TOR-regulated protein synthesis via phosphorylation of Raptor (45) and the TSC1-TSC2 complex (46). To delay progression through the cell cycle, AMPK activation decreases the expression of important cell cycle regulators (47) and induces stabilization of p53 and cyclin-dependent kinase inhibitors (48, 49). In cancer cells, AMPK has been shown to bestow tolerance to nutrient deprivation (50) and hypoxia (33) without restricting cell growth and proliferation. It has been proposed that AMPK promotes cancer cell survival by providing energy for essential cellular functions through processes such as fatty acid β-oxidation and/or autophagy (50, 51). The opposing effect of AMPK activation in normal and transformed cells could be due to differential deletion of downstream tumor suppressors allowing AMPK activation while mitigating the growth-limiting effect of the enzyme. For example, some cancers harbor TSC2 and/or p53 mutations, allowing AMPK activation without inhibition of protein synthesis or cell cycle arrest (52).

During the course of these experiments, reports suggesting that AICAR-induced AMPK activation inhibits proliferation, induces senescence, and promotes apoptosis in various cancer cells were published (53, 54). It was proposed that this was secondary to AMPK inhibition of anabolic processes such as protein synthesis (45, 46) or activation of p53 (48). Whereas AICAR is the best-characterized pharmacologic activator of AMPK and most of its effects have been prescribed to AMPK activation, AMPK-independent effects have been documented (26). In an attempt to compare their findings with ours, we treated human prostate cancer cells with AICAR. In our hands, millimolar concentrations of AICAR induce S-phase arrest and senescence independent of AMPK activation (data not shown). These data suggest that decreased proliferation in response to AICAR is not secondary to AMPK activation but a nonspecific effect of AICAR on nucleotide metabolism (26). New selective AMPK activators (55) may help to further define the role of AMPK in cancer therapy.

Protein kinase signaling pathways important in maintaining cell proliferation and survival under stressed conditions may provide the critical growth signals for premalignant lesions to progress to clinical prostate cancer. We show that AMPK is activated in primary prostate cancers and may
promote prostate cancer proliferation and survival. The prevalent detection of activated AMPK in primary human prostate cancer specimens indicates that AMPK is a potential candidate molecular target for chemoprevention of prostate cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


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