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Survival Advantage of AMPK Activation to Androgen-Independent Prostate Cancer Cells During Energy Stress

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Abstract

Androgen-independent prostate cancer usually develops as a relapse following androgen ablation therapy. Removing androgen systemically causes vascular degeneration and nutrient depletion of the prostate tumor tissue. The fact that the malignancy later evolves to androgen-independence suggests that some cancer cells are able to survive the challenge of energy/nutrient deprivation. AMP-activated protein kinase (AMPK) is an important manager of energy stress. The present study was designed to investigate the role of AMPK in contributing to the survival of the androgen-independent phenotype. Most of the experiments were carried out in the androgen-dependent LNCaP cells and the androgen-independent C4-2 cells. These two cell lines have the same genetic background, since the C4-2 line is derived from the LNCaP line. Glucose deprivation (GD) was instituted to model energy stress encountered by these cells. The key findings are as follows. First, the activation of AMPK by GD was much stronger in C4-2 cells than in LNCaP cells, and the robustness of AMPK activation was correlated favorably with cell viability. Second, the response of AMPK was specific to energy deficiency rather than to amino acid deficiency. The activation of AMPK by GD was functional, as demonstrated by appropriate phosphorylation changes of mTOR and mTOR downstream substrates. Third, blocking AMPK activation by chemical inhibitor or dominant negative AMPK led to increased apoptotic cell death. The observation that similar results were found in other androgen-independent prostate cancer cell

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lines, including CW22Rv1 abd VCaP, provided further assurance that AMPK is a facilitator on the road to androgen-independence of prostate cancer cells.

Keywords

AMPK; mTOR; prostate cancer; glucose deprivation

1. Introduction

Solid tumors are often characterized by a disorganized vasculature, which tends to hinder the delivery of oxygen and nutrients to the cancer cells [1,2]. In order to adapt to this hostile microenvironment, cancer cells need to develop a mechanism so that they may continue to thrive despite the challenge of hypoxia and nutrient deficiency. AMP-activated protein kinase (AMPK) is an important energy sensor whose main function is to block ATP-consuming processes and stimulate ATP-producing processes by phosphorylating rate-limiting enzymes associated with metabolic pathways and modifying signal transduction cascades [3,4]. In times of hypoxia or glucose deprivation, AMPK is activated to conserve or restore cellular ATP for survival [5,6]. Some examples of AMPK-mediated effects include suppression of mammalian target of rapamycin (mTOR) signaling to reduce protein synthesis, and promotion of fatty acid oxidation and glycosis [7,8].

Immunohistochemical analysis of hypoxia markers [9] and molecular imaging [10] lend support to the conclusion that prostate tumors, like many solid tumors in other organ sites, are afflicted by poor blood flow and unstable oxygenation. Consistent with these findings is the observation that AMPK is highly expressed in about 40% of human prostate cancer specimens [11], suggesting that a sizable proportion of them is under metabolic stress. However, the role of AMPK is controversial in prostate cancer cell studies in vitro in which cells are cultured in a normal condition, i.e. in the absence of either hypoxia or nutrient depletion. In one study, the down-regulation of AMPK by either small interfering RNA or chemical inhibitor was shown to decrease cell growth [11]. In contrast, other studies reported that a similar decrease in growth was achieved by activating AMPK with pharmacological agents [8]. As a whole, these in vitro studies are difficult to interpret because the effect of AMPK might have been distorted or masked by other factors which are more dominant than AMPK when cells are exposed to normoxia and when there is no lack of nutrients.

Androgen ablation therapy is commonly employed in the treatment of advanced prostate cancer. Signs of vascular degeneration, hypoxia, and metabolic stress in the prostate tumor tissue are exacerbated following surgical or medical castration [12]. After a short remission period, the majority of prostate cancer begins to progress and becomes androgen-independent. Clearly a subset of cells is able to survive the low oxygen and nutrient environment and emerge with a different phenotype. The present study was designed to investigate the role of AMPK in contributing to the development of androgen-independent prostate cancer. Glucose deprivation was instituted to model the metabolic stress encountered by these cells so that the significance of AMPK can be put into the proper context. Most of the experiments were carried out in both the androgen-dependent LNCaP

cells and the androgen-independent C4-2 cells. These two cell lines have the same genetic background, since the C4-2 line is derived from the LNCaP line [13]. In order to address the issue of lineage specificity, additional experiments were also conducted in two other androgen-independent cell models: CW22Rv1 and VCaP [14].

2. Materials and methods

2.1. Cell cultures

LNCaP, CW22Rv1 and VCaP prostate cancer cell lines were obtained from the American Type Culture Collection, Manassas, VA. C4-2 cells were provided by Dr. Leland Chung (Department of Urology, Emory University School of Medicine, Atlanta, GA). All cells, except VCaP, were cultured in RPMI 1640 medium. VCaP cells were cultured in DMEM as recommended by ATCC. Both types of media were supplemented with 10% fetal bovine serum, 2 mmol/L of glutamine, 100 units/mL of penicillin, and 100 μg/mL of streptomycin. The cultures were maintained at 37°C in an atmosphere of 5% CO_2 and 95% air. Cells with <10 passage number were used in all experiments.

2.2. Glucose or amino acid deprivation

In all except one experiment, the cultures were subjected to glucose deprivation. This was achieved by replacing the regular RPMI 1640 medium with a glucose-free RPMI 1640 medium from Invitrogen (Carlsbad, CA). For the amino acid deprivation experiment, cells pre-cultured in complete regular medium were subjected to the L-glutamine and L-leucine free RPMI medium (MP Biochemicals) for the required duration of the experiment.

2.3. Cell survival and cell death analysis

Analysis of cell survival after glucose deprivation (GD) was performed by using the MTT assay as described in our previous publication [15]. The data are expressed as percent surviving cells after GD. Untreated cells served as the control in every experiment. Total cell death (both necrotic and apoptotic cell death) determination was done by flow cytometric analysis. Cells were stained with propidium iodide (PI, 5μg/ml) according to the method described in previous publications [16,17]. This method offers the advantage of providing quantitative data. The fluorescence of PI was captured with a 585 nm filter in a FACScan flow cytometer (Becton Dickinson). Both dot plot and histogram analyses were performed using the FCS Express Software in a population of 20,000 cells. Total cell death was calculated as the percent of PI-positive nuclear staining cells. The results in the treatment group are expressed as fold of change compared to the control. In the experiments with transfected siRNA, the cells were harvested with trypsin solution at 24 and 48 h after GD. Cell counts were performed in triplicates with a hemocytometer using trypan blue (0.2%) exclusion to identify viable cells. Non-viable (positively-stained) cells were calculated as a percent of total.

2.4 LDH Release as a Marker of Necrosis

Cell necrosis was estimated by determining lactate dehydrogenase (LDH) release in the medium [18] in which cells were cultured with or without glucose for 48 h. The activity of LDH was measured by the CytoTox 96 Cytotoxicity Assay Kit (Promega, Madison, WI)

according to manufacturer's protocol. The quantitation of LDH in the treatment group is expressed as fold of change compared to the control.

2.5. Chemical inhibition of AMPK activation

Compound C, obtained from Calbiochem, was used to inhibit AMPK activation at a concentration of 10 μM. DMSO was added as the vehicle for the untreated control.

2.6. Dominant negative AMPK expression

A dominant negative form of AMPK (DN-AMPKα1) was generated originally by David Carling and co-workers as described previously [19]. The DN-AMPKα coding regions were sub-cloned into pcDNA-Neo to generate pcDNA-Neo-DN-AMPKα1. The construct was obtained as a gift from Dr. Jill Suttles (University of Louisville School of Medicine, Louisville, KY). The mycDN-AMPKα1, which encodes N-terminal Myc-tag with DN-AMPKα1 cloned in pCDNA3, was obtained from Dr. David Carling's laboratory (Hammersmith Hospital, London, UK). Prostate cancer cells were transiently transfected with Lipofectamine 2000 according to the manufacturer's instructions.

2.7. AMPKα1 Knockdown by siRNA

All materials for siRNA transfection were purchased from Ambion (Austin, TX). Transient transfection of siRNA was done using a protocol recommended by the manufacturer. The AMPKα1 siRNA sequences, which were accessed from the Ambion pre-designed siRNA library (ID number s100), were as follows: sense: 5′-GGAUCCAUCAUAUAGUUCAtt-3′, antisense: 5′-UGAACUAUAUGAUGGAUCCtc-3′. Non-silencing siRNA sequence was used as the negative control. All siRNAs, obtained in lyophilized and annealed form, were resuspended in diethylpyrocarbonate-treated distilled water to achieve a stock concentration of 20 μM, and stored at −20°C in 50-μl aliquots. C4-2 cells were plated onto 6-well plates to achieve 60% confluency. Lipofectamine 2000 transfection reagent (Invitrogen) and fresh medium containing 40 nM of either negative control or AMPKα1 siRNA were incubated in separate tubes for 5 min. The transfection reagent and siRNA solution were then combined and incubated for 25 min at room temperature to allow complex formation. The siRNA-transfection reagent complexes were added drop-wise to each well of cells bathed in 2 ml of fresh medium. Transfected cells were cultured in a glucose deprived condition for 48 h, and harvested for survival and Western blot analysis.

2.8. Western blot analysis

Equal amounts of protein were analyzed in duplicate by SDS-PAGE. Protein concentrations were measured by the BCA protein assay kit as per manufacturer's protocol (Pierce). Antibodies to AMPKα, phospho-AMPKα (Thr172), mTOR, phospho-mTOR (Thr2446 and Ser2448), p70S6K, phospho-p70S6K (Ser371), S6, phospho-S6 (Ser235/236), Myc-tag and PARP were purchased from Cell Signaling Technology (Beverly, MA). Anti–β-actin was obtained from Sigma and anti-GAPDH from Santa Cruz Biotechnology. Immunoreactive proteins were detected with a HRP-conjugated secondary antibody (Biorad) and visualized by using an enhanced chemiluminescence detection system (Amersham Bioscience).

2.9. Statistical analysis

The Student t-test was used to determine statistical difference between treatment and control values. A P value of <0.05 is considered significant.

3. Results

3.1. Enhanced survival and AMPK activation in C4-2 cells following glucose deprivation (GD)

LNCaP (androgen-dependent) and C4-2 (androgen-independent) prostate cancer cells were subjected to GD for 5 consecutive days. No cell growth was observed during this period. The effect of GD on cell survival, as determined by the MTT assay, is shown in Fig. 1A. Cells remained mostly viable after 1 day of GD. By day 5, about 60% of LNCaP cells did not survive, as opposed to only 25% of C4-2 cells, suggesting that C4-2 cells are more resistant to GD-induced cell death.

Since GD is known to cause phosphorylated AMPK activation, the protein level of phospho-AMPKα was determined by Western blotting at 8, 16 and 24 h after GD (Fig. 1B). These early time points were chosen in order to detect the changes that occurred before the onset of cell death. The results showed that phospho-AMPKα increased at a much faster rate and reached a higher level in C4-2 cells than in LNCaP cells. The total amount of AMPKα was unchanged in either cell type.

For comparison with GD, the effect of amino acid deprivation (AAD) on cell growth was examined in a second experiment (Fig. 1C). Both LNCaP and C4-2 cells were able to grow in the presence of AAD, although at a slower pace than the control culture in complete medium. However, AAD caused a similar percentage of growth inhibition in both cell types. Thus the survival advantage of C4-2 cells over LNCaP cells with GD is not reproduced with AAD. There were only minor changes in phospho-AMPKα level in either cell type following AAD (Fig. 1D). The above data suggest that AMPK activation responds specifically to energy deficiency rather than to amino acid or nutrient deficiency. GD removes a major source of energy for maintaining cellular functions, whereas cells can breakdown non-essential proteins to recycle amino acids during AAD.

3.2. Functional analysis of AMPK activation following glucose deprivation (GD)

Two direct phosphorylation targets of AMPK, acetyl CoA carboxylase (ACC) and mammalian target of rapamycin (mTOR), were examined following GD. The same time course was set up for this experiment as for the AMPK activation experiment. ACC is a key enzyme in the pathway of fatty acid biosynthesis and is inactivated by phosphorylation. Inhibiting ACC activity is an energy saving measure. The increase of phospho-ACC was more rapid and robust in C4-2 cells than in LNCaP cells (Fig. 2A). Thus the rate and magnitude of ACC phosphorylation paralleled closely the pattern of AMPK activation in each cell type. AMPK modifies mTOR phosphorylation at two different sites. It promotes phosphorylation at Thr2446 and inhibits phosphorylation at Ser2448 [20,21]. In LNCaP cells, phosphorylation at Thr2446 increased 1.8 fold at 24 h, but phosphorylation at Ser2448 remained fairly stable (Fig. 2A). In C4-2 cells, phosphorylation increased at Thr2446 and

decreased at Ser2448 at a much faster pace. The trend is in line with the steeper rise of AMPK activation in this cell model.

mTOR plays an important role in any cell stress situation [22]. The phosphorylation changes of mTOR caused by AMPK result in a lower activity of mTOR. Next, the activity of mTOR was assessed by determining the phosphorylation status of two target substrates: S6 and p70S6K (Fig. 2B). Following GD, the decrease of both phospho-S6 and phospho-p70S6K occurred earlier in C4-2 cells than in LNCaP cells, suggesting that the down-regulation of mTOR activity was functional.

3.3. Inhibiting AMPK activation decreases survivability of C4-2 cells following glucose deprivation (GD)

In order to address the significance of AMPK activation to cell survival in an energy-stressed environment, an experiment was performed to study survival response when AMPK activation was blocked. Compound C is a small molecule inhibitor of AMPK activation [11]. Phospho-AMPKα changes in LNCaP and C4-2 cells at 72 h following GD and in the absence or presence of Compound C are shown in Fig. 3A. In LNCaP cells, the increase of phospho-AMPKα, which was detectable at earlier time points, was no longer evident at 72 h after GD. Compound C had minimal effect on baseline level of AMPK activation in LNCaP cells. In contrast, C4-2 cells still maintained a higher level of phospho-AMPKα at 72 h after GD. In the presence of Compound C, phospho-AMPKα was reduced to near baseline level. More importantly, 75% of C4-2 cells did not survive the GD condition compared to 40% of LNCaP cells when Compound C was present in the culture (Fig. 3B). The data therefore suggest that AMPK activation is critical to the resistance of C4-2 cells against energy deprivation.

3.4. Dominant negative AMPK (DN-AMPKα) expression augments cell death of glucose-deprived C4-2 cells

The expression of a DN-AMPKα1 construct in C4-2 cells resulted in a marked down-regulation of phospho-AMPKα at both 24 and 48 h (Fig. 4A). Total AMPKα increased slightly in the transfected cells because the AMPKα antibody also recognizes the DN-AMPKα. As expected, the loss of AMPKα activation due to the DN-AMPKα led to a decrease of phospho-ACC and phospho-mTOR (Thr2446), and an increase of phospho-mTOR (Ser2448). The ACC and mTOR data, which are shown in Fig. 4A, confirmed that the DN-AMPKα construct was exerting its intended biochemical effects downstream.

In terms of survival response to GD, the expression of the DN-AMPKα caused more cells to die, as determined by nuclear propidium iodide staining of dead cells (Fig. 4B). With the loss of AMPK activation, there was a 4-fold and 2.3-fold increase of total cell death at 24 h and 48 h, respectively. It was not possible to do the cell death experiment past the 48 h time point because of the transient expression of the DN-AMPKα.

In order to confirm the successful transfection of the DN-AMPKα, we used the myc-tagged AMPKα plasmid and tracked the expression of myc by Western blot analysis (Fig. 4C). The results show that myc was detected only in the mycDN-AMPKα transfected cells. Similar to

the data shown in Fig. 4A, total AMPKα protein was increased in the transfected cells, but there was a decrease of phospho-AMPKα.

3.5. siRNA mediated AMPKα knockdown enhances cell death of glucose-deprived C4-2 cells

In addition to the use of DN-AMPKα, we also carried out another experiment with siRNA knockdown of AMPKα expression in C4-2 cells undergoing glucose deprivation. The data in Fig. 5A show that total AMPKα was decreased in the siRNA-transfected cells, and so were phospho-AMPKα, phospho-ACC and phospho-mTOR (Thr2446). As expected, the opposite effect was observed with phospho-mTOR (Ser2448). The trypan blue exclusion assay demonstrated that cell death due to GD was significantly increased when AMPK activation was jeopardized by RNA interference (Fig. 5B). These observations clearly indicate that AMPK activation is important for survival of the C4-2 cells under GD.

3.6. Resistance of other androgen-independent prostate cancer cells to glucose deprivation-induced cell death and the reversal of this effect by DN-AMPKα

Two other human androgen-independent prostate cancer cell lines, CW22Rv1 and VCaP, were studied with respect to their sensitivity to GD. Compared to LNCaP cells, both CW22Rv1 and VCaP cells showed greater survivability in the absence of glucose (Fig. 6A). Only 25% of LNCaP cells survived GD, as opposed to 45% of CW22Rv1 cells and 85% of VCaP cells. Consistent with the finding in C4-2 cells, the expression of the DN-AMPKα in CW22Rv1 and VCaP cells also caused more cell death (Fig. 6B). The increase was 1.6-fold in CW22Rv1 cells and 3-fold in VCaP cells. Both CW22Rv1 and VCaP cells showed robust and persistent increases of AMPK activation following GD (Fig. 6C). The knockdown of phospho-AMPKα by the DN-AMPKα, as confirmed by Western blotting, also correlated well with increased cell death in both models.

3.7. AMPK inhibition causes enhanced apoptotic cell death in a glucose-deprived condition

Glucose deprivation may lead to two types of cell death, necrosis or apoptosis [23,24]. Cell death detection by propidium iodide staining does not distinguish between these two types. An experiment was carried out to determine whether GD caused necrosis and whether AMPK inhibition by the DN-AMPKα produced more necrotic or apoptotic cell death. The LDH release assay was used to assess the extent of necrosis, and the results are expressed as fold of change compared to the value obtained in control cultures without GD (Fig. 7A). There was a 6-fold increase in C4-2 cells, an 8-fold increase in CW22Rv1 cells, and a 2-fold increase in VCaP cells after 48 h of GD. It should be noted that the amount of LDH release due to necrosis is cell specific, and that the magnitude of the increase of LDH release cannot be equated directly to the magnitude of cell death induction. Expression of the DN-AMPKα in these cells did not lead to a further increase of LDH release even though total cell death was increased as seen in an earlier experiment (Fig. 6B). The inference is that inhibiting AMPK activation causes cells to die by apoptosis.

To test the above hypothesis, PARP cleavage, which is a hallmark of apoptotic cell death [25], was used to assess the extent of apoptosis under various treatment conditions. C4-2 and

CW22Rv1 cells were subjected to GD with or without DN-AMPKα expression. There was little PARP cleavage at 18 h after GD, but the cleaved PARP was definitely evident at 36 h in both cell lines (Fig. 7B). Inhibiting AMPK activation by the DN-AMPKα resulted in a 1.7-fold increase of PARP cleavage in C4-2 cells, and a 1.8-fold increase in CW22Rv1 cells. The results suggest that the major mode of cell death induction by down-regulating AMPK activation is accounted for by apoptosis.

4. Discussion

A major conclusion of the present study is that AMPK activation offers a survival advantage to androgen-independent prostate cancer cells in an energy-starved microenvironment. The conclusion is underlined by two key findings. First, the activation of AMPK by glucose deprivation is much stronger in androgen-independent than in androgen-dependent prostate cancer cells, and the robustness of AMPK activation is correlated favorably with cell viability. Second, blocking AMPK activation by chemical inhibitor or dominant negative AMPK leads to increased cell death. Androgen-independent prostate cancer, which is very resistant to chemotherapy, usually develops as a relapse following androgen ablation therapy. Since androgen is critically involved in maintaining vascular integrity of prostate tumor tissue [12] and regulating the expression of nutrient transporters in prostate cancer cells [26], removing androgen systemically by medical or surgical castration would be expected to lead to nutrient depletion and consequently, to regression of the malignancy. The fact that the malignancy later evolves to androgen-independence implies that not all the cancer cells are eradicated by androgen ablation. Some cells are able to survive the insult of energy/nutrient depletion and continue to propagate. The present findings suggest that AMPK may help cells overcome the challenge of energy deprivation.

The LNCaP and C4-2 cell lines used in this study are appropriate models to reconstruct the molecular events associated with the transition from androgen-dependence to androgen-independence. The C4-2 cell line is derived from a recurrent LNCaP xenograft after castration of the host animal [13]. C4-2 cells have therefore undergone a selection process and have acquired the ability to withstand a nutrient starvation crisis. The observation of a greater AMPK activation in C4-2 cells than in the parental LNCaP cells is supportive of the role of AMPK in contributing to the development of a more aggressive pathology. The fact that similar results were found in other androgen-independent prostate cancer cell lines, including CW22Rv1 and VCaP, provides further assurance that AMPK is a facilitator on the road to androgen-independence. It is apparent that these cells, after gaining androgen-independence, still retain certain signature phenotype which has given them the resilience to rebound from episodes of energy deprivation.

AMPK is a crucial manager of energy stress [5]. One main function of AMPK is to inhibit mTOR activity so that protein synthesis is limited to a minimum [27,28]. AMPK activation by low glucose and oxygen has been shown to suppress mTOR activity even in the presence of full growth factors and active Akt and Erk signaling [21,28,29]. This is an energy conservation measure. When faced with an energy crisis, cells need to put survival as a priority before expending energy for proliferation. The pro-survival effect of down-regulating mTOR in stress situations has been reported previously [30]. AMPK is also

known to stimulate autophagy [31]. In times of energy stress, autophagy may be considered as a way to recycle nutrients in order to support essential cellular functions [31,32]. AMPK activation may also mediate the recruitment of glucose transporters to the cell membrane [3,33]. This effect is particularly relevant to prostate cancer cells following androgen ablation because of diminished nutrient delivery as a result of vascular disintegration. Additionally, AMPK contributes to the Warburg effect of anaerobic metabolism (necessitated by hypoxic condition in the tumor) by favoring glycosis over the Krebs cycle in generating ATP [3].

In summary, AMPK allows prostate cancer cells to remain viable when they suddenly lose the fountain of sustenance fueled by the androgen signal. Cells which are successful in achieving a vibrant AMPK activation phenotype would be better equipped for transition to androgen-independence. AMPK is thus a legitimate target of intervention to block the development of androgen-independent prostate cancer. The induction of apoptotic, rather than necrotic, cell death by abrogating AMPK activation, as demonstrated in the present study, is advantageous clinically. Tumor necrosis often provokes inflammation and cytokine response that may encourage growth of the surviving cancer cells [24]. Denying prostate cancer cells the ability to adapt to metabolic stress and increasing the rate of cell killing at the time of androgen ablation therapy would be a worthwhile strategy to pursue in controlling androgen-independent prostate cancer.

5. Conclusion

The present study provides evidence to support the conclusion that AMP-activated protein kinase (AMPK) offers a survival advantage to prostate cancer cells in an energy-starved condition that is often experienced following androgen ablation therapy. Cells which are successful in achieving a vibrant AMPK activation phenotype are better equipped for transition to androgen-independence. AMPK is thus a legitimate target of intervention to block the development of androgen-independent prostate cancer.

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Abbreviations

ACC	acetyl coenzyme A carboxylase
AAD	amino acid deprivation
AMPK	AMP-activated protein kinase
Comp C	Compound C
DN	dominant negative
GD	glucose deprivation
GADPH	glyceraldehyde 3-phosphate dehydrogenase

LDH	lactate dehydrogenase
mTOR	mammalian target of rapamycin
PARP	poly ADP-ribose polymerase
SD	standard deviation
siRNA	small interference RNA

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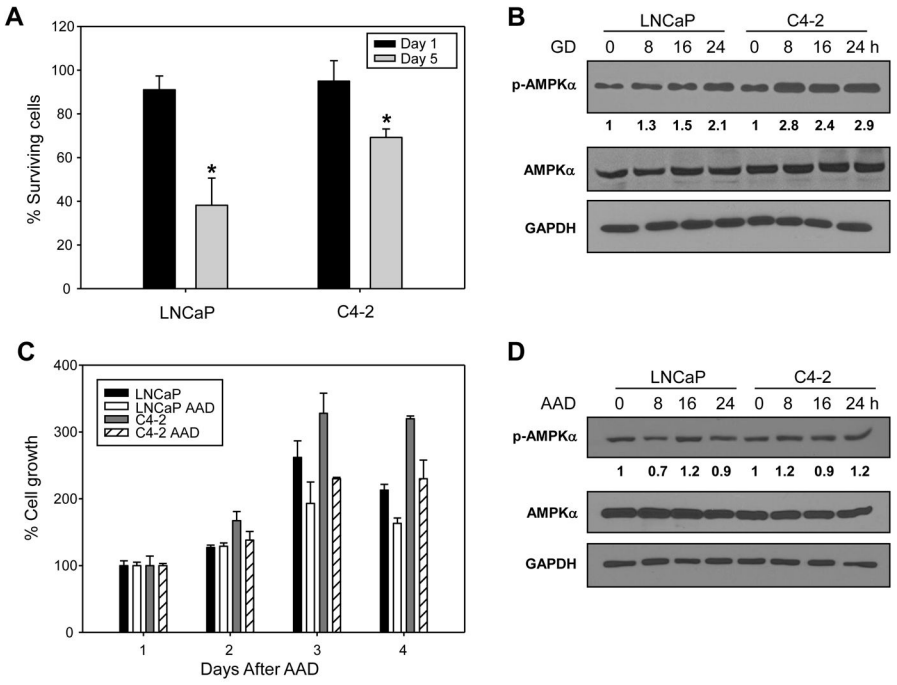

Fig. 1.

Effect of glucose or amino acid deprivation on cell survival and AMPK activation in LNCaP and C4-2 cells. (A) MTT cell survival data following glucose deprivation. The results (mean ± SD, n=3) are expressed as % surviving cells compared to the value of the untreated control, which is set as 100%. *significantly different from each other, $P<0.05$. (B) Western blot of phosphorylated or total AMPK in cells following glucose deprivation (GD). (C) MTT cell growth data following amino acid deprivation (AAD). (D) Western blot of phosphorylated or total AMPK following AAD.

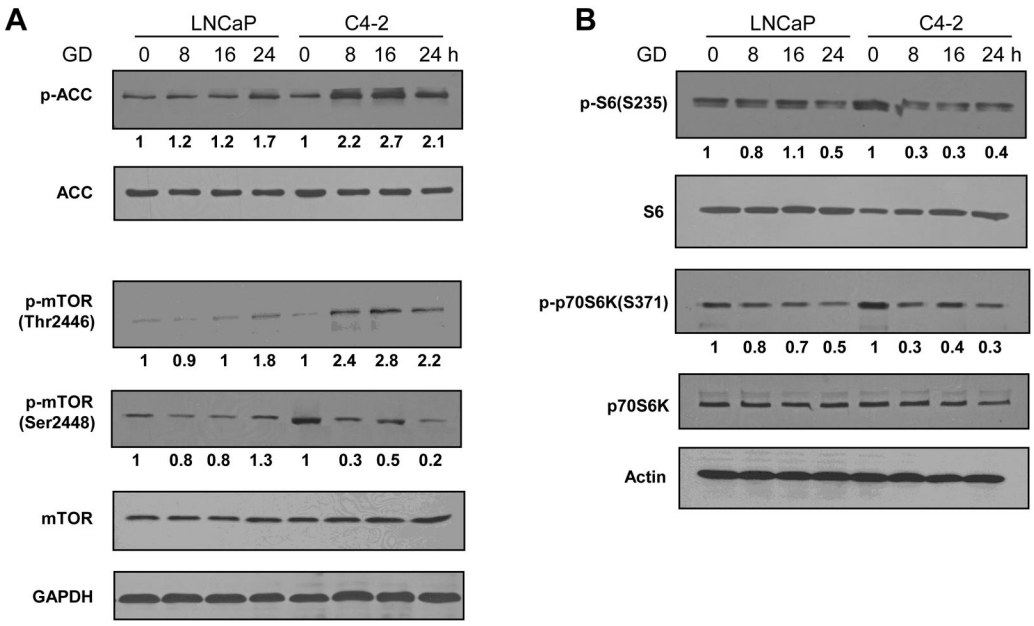

Fig. 2.

Effect of glucose deprivation (GD) on phospho-ACC, phospho-mTOR, phospho-S6 and phospho-p70S6K. The substrates in (A) are the direct targets of AMPK, while the substrates in (B) are the direct targets of mTOR.

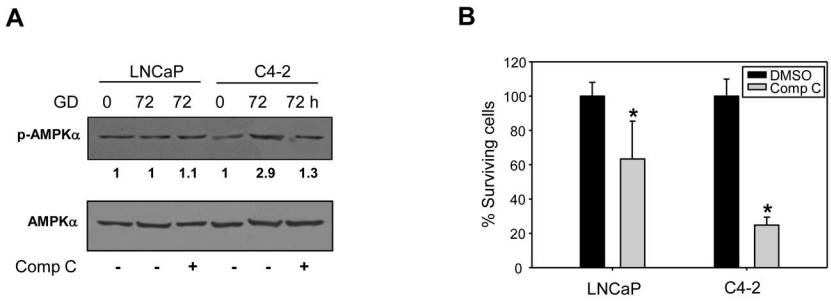

Fig. 3.

Effect of Compound C on AMPK activation (A) and cell survival (B) in LNCaP and C4-2 cells subjected to glucose deprivation. The cell survival data were obtained at day 3. The results from Compound C-treated cells are expressed as a percentage of the value from DMSO (vehicle) treated cells (set as 100%). *significantly different from each other, $P<0.05$.

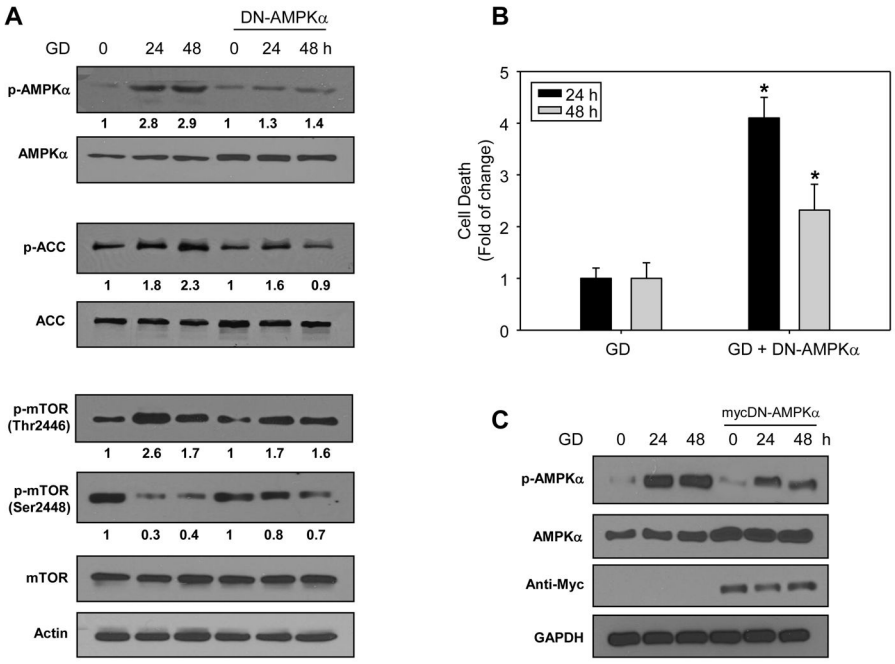

Fig. 4.

Effect of dominant negative AMPK (DN-AMPKα) on AMPK activity (A) and cell death (B) in C4-2 cells following glucose deprivation (GD). (A) Western blot of phospho-AMPK, phospho-ACC, and phospho-mTOR. (B) Cell death data as determined by flow cytometric analysis of propidium iodide-stained cells. Effect of AMPK knockdown on cell death is expressed as fold of increase compared to the value observed in the absence of knockdown. *significantly different than the GD only value, $P<0.05$. (C) Western blot of phospho-AMPK, total AMPK and myc of mycDN-AMPKα transfected cells.

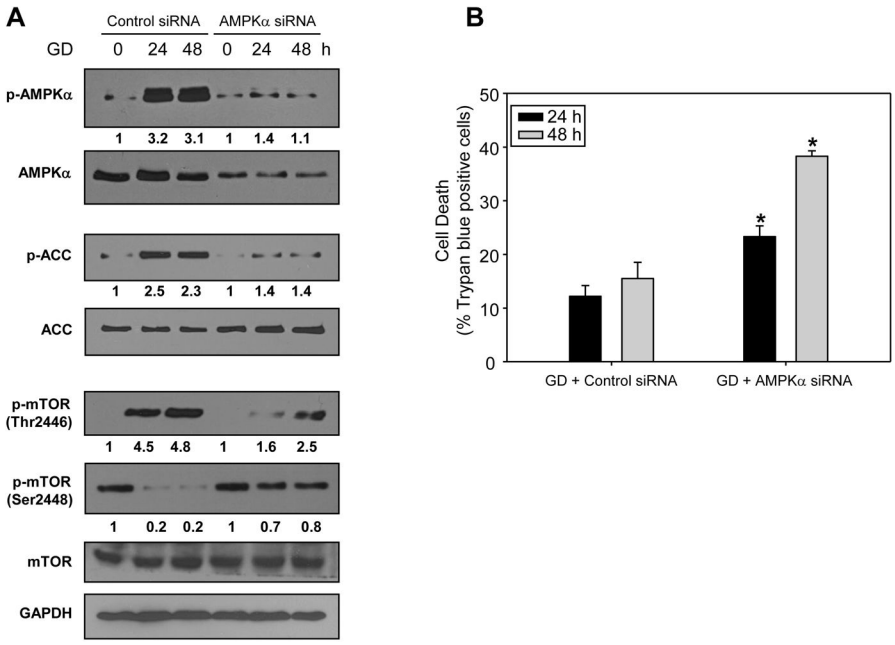

Fig. 5.

Effect of AMPK-siRNA on AMPK activity (A) and cell death (B) in C4-2 cells following glucose deprivation (GD). (A) Western blot of phospho-AMPK, phospho-ACC, and phospho-mTOR. (B) Cell death data as determined by trypan blue exclusion analysis. Effect of AMPK knockdown on cell death is expressed as percent of trypan blue positive cells. *significantly different than the GD only value, $P<0.05$.

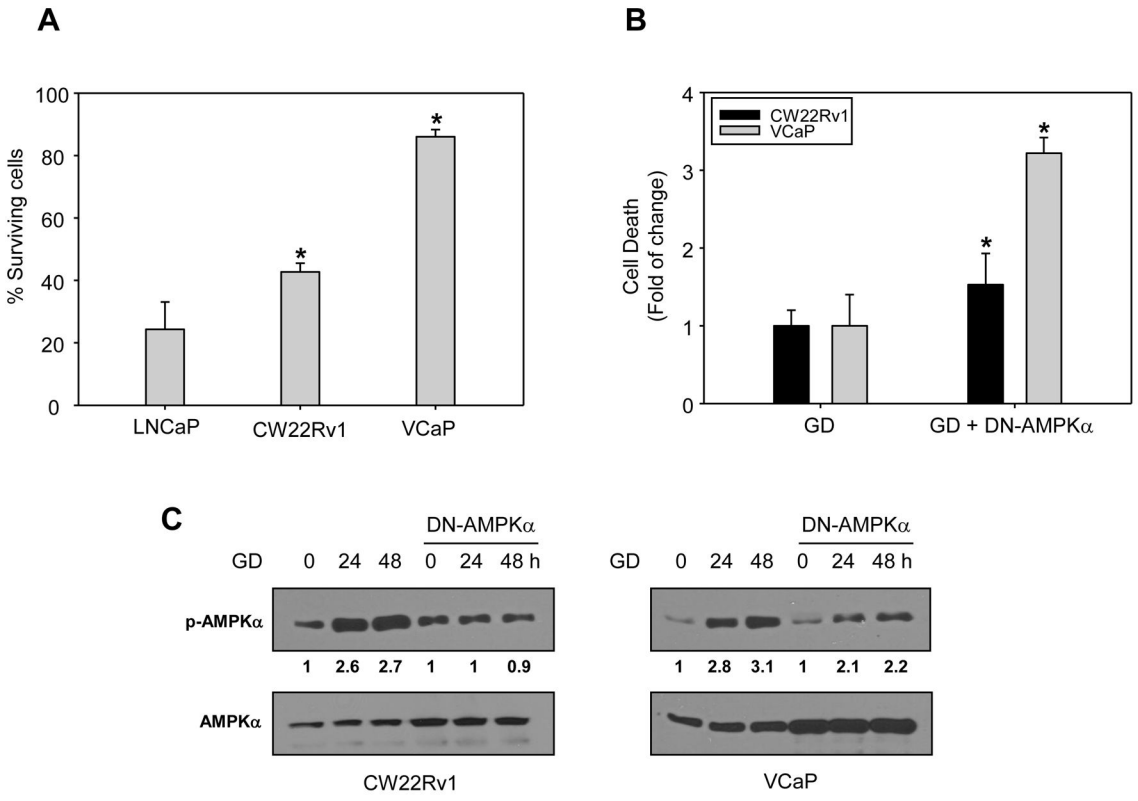

Fig. 6.

Effect of glucose deprivation (GD) on cell survival and cell death in CW22Rv1 and VCaP cells with or without AMPK activation knockdown by DN-AMPKα. (A) MTT cell survival data after 5 days of GD. The results are expressed as % surviving cells compared to the value of the untreated control, which is set as 100%. *significantly different than the LNCaP value, $P<0.05$. (B) Cell death data as determined by flow cytometric analysis of propidium iodide-stained cells. The effect of AMPK activation knockdown on cell death is expressed as fold of increase compared to the value observed in the absence of knockdown. *significantly different than the GD only value, $P<0.05$. (C) Confirmation of decreases of phospho-AMPKα by DN-AMPKα.

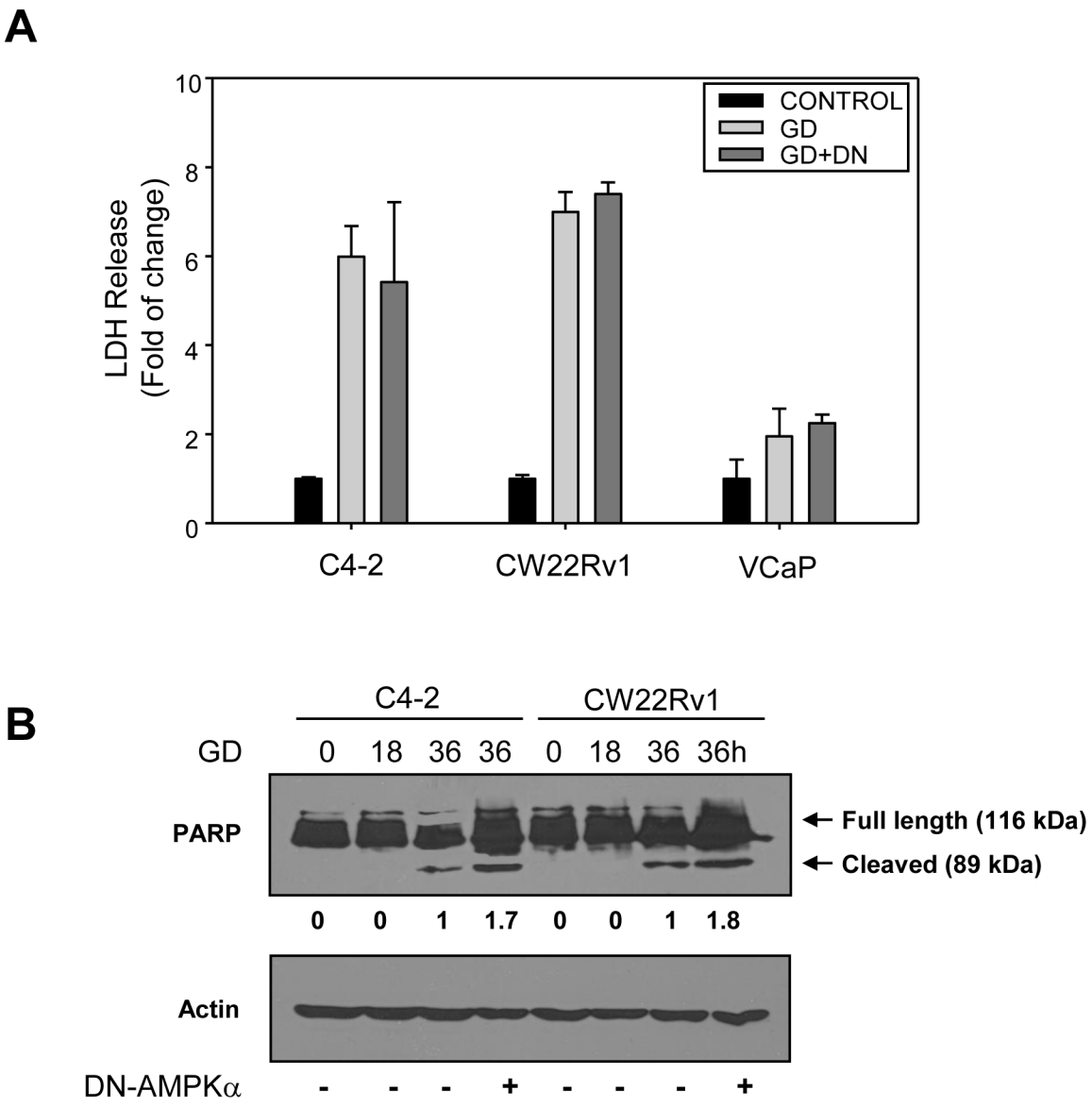

Fig. 7.

Effect of AMPK knockdown by DN-AMPKα on cell death induction following glucose deprivation (GD). (A) LDH release as an indicator of necrotic cell death. The data are expressed as fold of increase compared to the value from untreated control cells (set as 1). (B) PARP cleavage as an indicator of apoptotic cell death. The Western blot shows both full length and cleaved PARP with or without DN-AMPKα transfection.