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Activation of AMPK promotes thyroid cancer cell migration through its interaction with PKM2 and β-catenin

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ABSTRACT

AMP-activated protein kinase (AMPK) is induced by the exhaustion of cellular energy and activates adaptive alterations in cellular metabolism, which is the basis for cell survival during different environmental stresses. We aimed to investigate the biological functions of AMPK and its molecular mechanism in regulating thyroid cancer (TC) progression. In current study, we found that activation of AMPK by multiple agonists suppresses TC cell proliferation. However, AMPK activation also led to TC cell migration at the same time. Depletion of AMPK abolished the effect of its agonist on cell multiplication and migration. Mechanistic investigations revealed that the impact of AMPK in terms of cell migration is dependent on its nuclear translocation, since site mutation of AMPK in its nuclear translocation domain (K244A) abolished TC cell migration but did not affect the inhibition of cell proliferation by AMPK agonist. Moreover, the nuclear AMPK recruits PKM2 and β-catenin by their interaction, which promotes the transcription of cell migration related genes, including MMP7 and c-Myc. Furthermore, depletion of PKM2/β-catenin abolished the migration effect of AMPK agonists, but did not affect their effects on suppression of cell proliferation. Our results provided a novel function of AMPK in cancer migration, and suggested that a combination of AMPK activation and PKM2 depletion or inhibition can be a new strategy to achieve better therapeutic effects for TC patients.

1. Introduction

Thyroid cancer (TC) is originated from thyroid follicular/parafollicular cells. Around the world, the incidence rate of TC increased dramatically in the most recent several years, causing the deaths of 36,000 patients in 2010, with an increase of 50% from 24,000 cases in 1990. Even with improving medical conditions and technology, its 5-year survival rate remains low [25]. The prevalence of TC has gone up by about 4.5% on average each year between 2007 and 2011 in America, and ranks as eighth most frequent cancer in China, which makes it as a substantial burden to public health [5,22]. TC is considered as a multicausal disease that are closely correlated to environmental and genetic factors, for instance, exposure to ionizing radiation, adiposity, genetic changes, and epigenetic modifications [19]. During the progression of thyroid malignancy, significant metabolic alterations have been recently described in addition to histological, cytological, and molecular abnormalities. TC possesses the ability to change metabolism from the oxidative to the glycolytic phenotype, which improves glycolysis to maintain rapid cell proliferation and tumor progression [9]. Therefore, targeted therapy towards the metabolism of cancer cells has arisen as a new way to prevent and cure thyroid cancers [21].

Recent epidemiological studies and laboratory experiments have indicated that the agonists of AMP-activated protein kinase (AMPK) activity, metformin (MF) and phenformin (PF), show an anti-cancer activity in TC treatment [20,21]. The role of AMPK as an energy modulator has been well established [11,14]. Under conditions of

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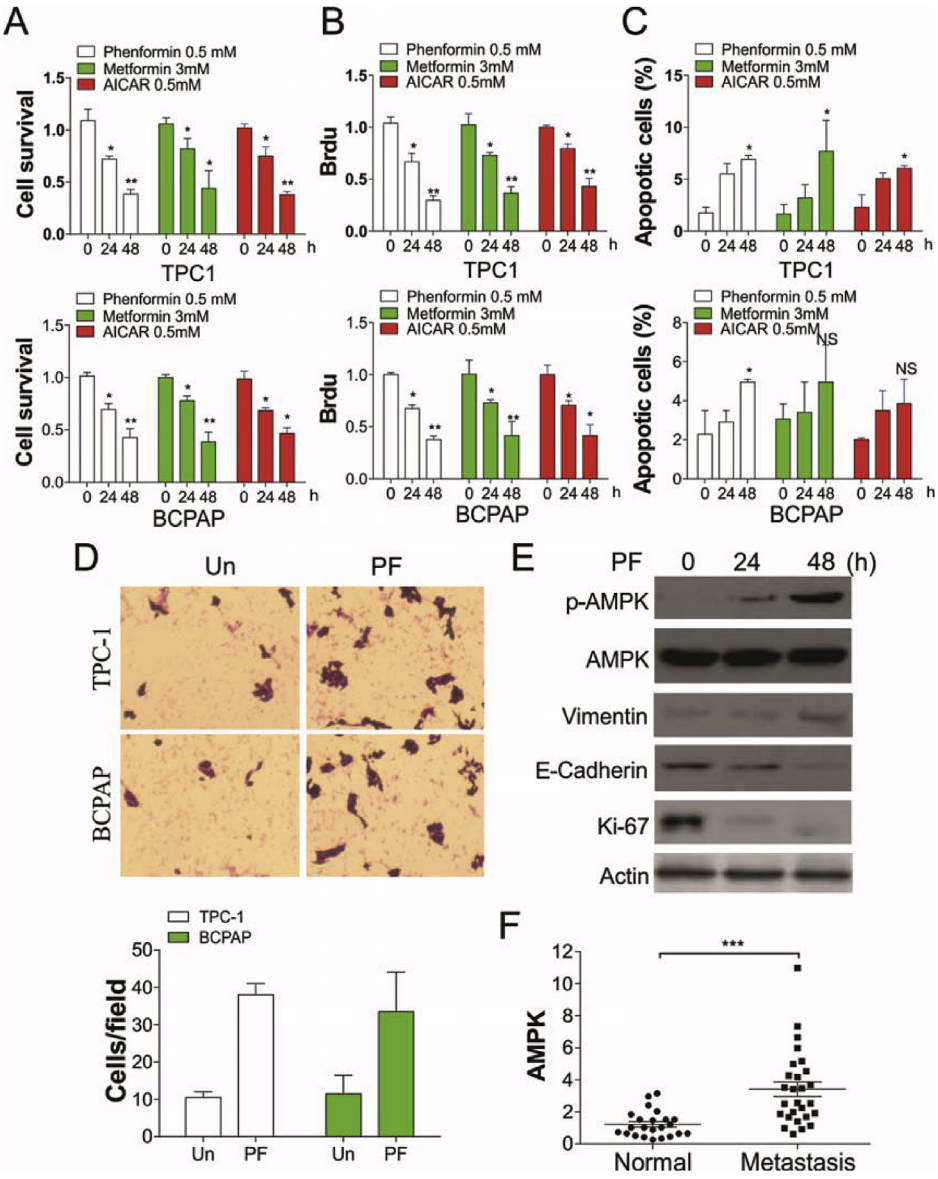

Fig. 1. Activation of AMPK suppressed TC cell proliferation but promoted cell migration. (A–C) TPC-1 and BCPAP cells were treated with phenformin (PF, 0.5 mM) or AICAR (0.5 mM), as well as metformin (MF, 3 mM) for the indicated time point. (A) The cell activity was analyzed via MTT assessment. (B) The cell proliferation was analyzed via BrdU assay. (C) The programmed cell death was analyzed via Hoechst 33342 staining. (D) TPC-1 and BCPAP cells was treated with PF for 48 h and cell migration was analyzed by a transwell migration assay. (E) Western blots for indicated proteins from PF-treated TPC-1 cells at the indicated times. Actin was used for normalization. (F) The protein expression level of AMPK within the thyroid tissues from normal TC patients (n = 23) or patients with metastasis (n = 27). One way ANOVA was used for A-C. Student's t-tests was used for statistic calculation for F. NS, $p > 0.05$, *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

nutrient deprivation and metabolic stress, activated AMPK acts to maintain intracellular energy homeostasis by down-regulating energy consuming (anabolic) pathway while in turn up-regulating energy producing (catabolic) pathway to replenish cellular ATP stores [12]. In addition to playing a vital role in regulating normal cell metabolism, evidences showed that inhibition or loss of AMPK activity may potentiate certain oncogenic signaling pathways underlying tumor cell growth and proliferation in some cancers [30]. For example, in differentiated and undifferentiated thyroid cancer cells, Chen et al. proved that in vitro activation of AMPK with metformin inhibited cell growth, stimulated cell cycle arrest and apoptosis, inhibited colony sphere formation, and chemosensitized cells to doxorubicin [4]. Furthermore, Choi et al. recorded that therapy with the nucleoside analog 5-aminoimidazole-4-carboxamide-ribonucleoside (AICAR), a direct AMPK stimulator, induced AMPK activation among BRAFV600E-mutant TC cell lines, leading to S-phase cell cycle arrest or apoptosis through the down-regulation of ERK as well as mTOR/p70S6K signaling [7]. However, despite promising results by using metformin and AICAR treatments in vitro and in *vivo*, their serum levels were too high for clinical use to obtain the ideal antitumor effects.

Although activation of AMPK suppresses tumor cell multiplication and energy metabolism, several reports showed a mediating function for AMPK over cancerous cell invasion. Lysophophatidic acid (LPA) could effectively activate AMPK and facilitate ovarian carcinoma metastasis [15]. Park et al. demonstrated that the capability of anthocyanins to restrain the metastatic phenotype of hepatoma carcinoma cells could be abolished through silenced AMPK [18]. All those results indicate that a critical role for AMPK in tumorous cell invasion and metastasis. However, the underlying mechanism of AMPK in terms of promoting tumor migration is still unknown. Our research results indicated that activated AMPK suppressed the TC cell proliferation, but enhanced the migratory ability of cells. Mechanistically, after activation, AMPK recruits PKM2 and β-catenin into the nucleus and triggers the transcription of several migration related genes, including MMP7 and c-Myc. Our results suggested that inhibition of PKM2 or β-catenin could be an alternative strategy to overcome the TC migration caused by AMPK activation.

2. Materials and methods

2.1. Patient samples

The clinic TC tumor samples were obtained from normal TC patients (n = 23) and patients with metastasis (n = 27) in RenJi hospital,

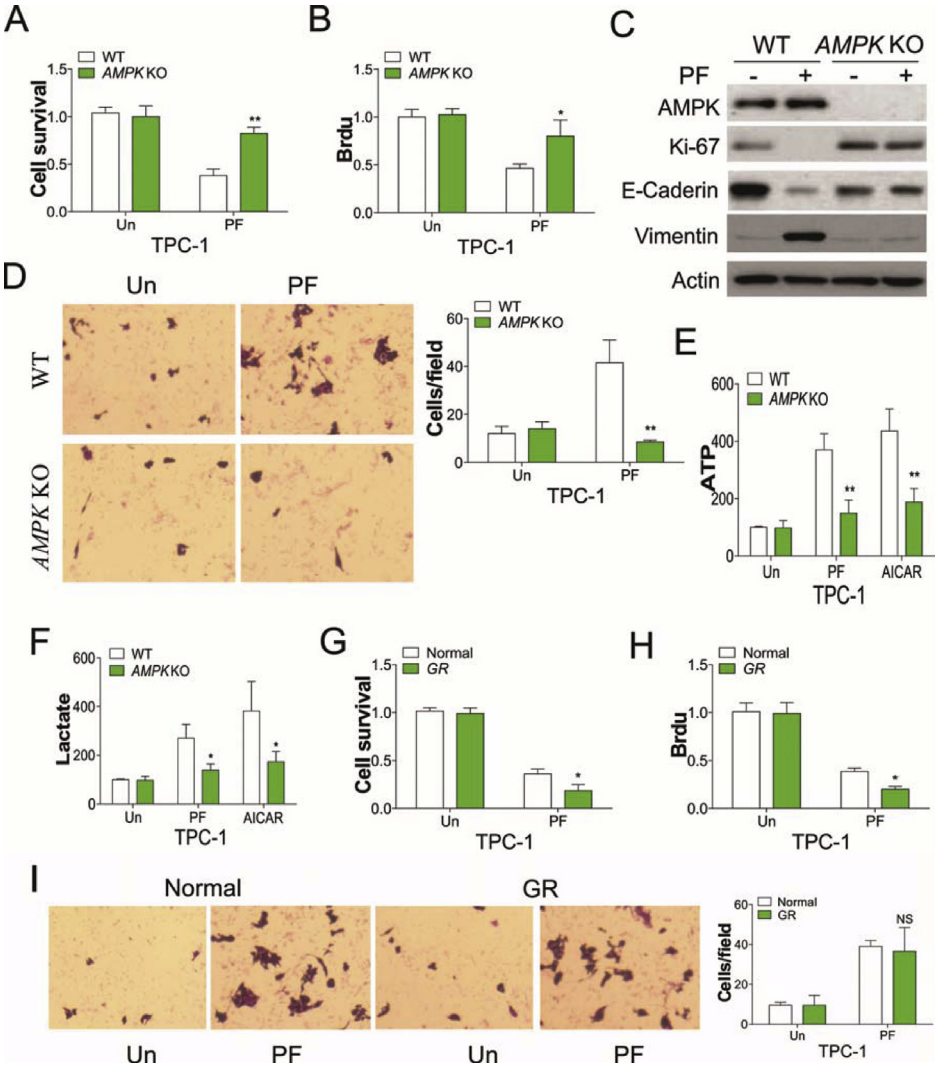

Fig. 2. AMPK mediates multiplication and migration of TC cells. (A) Cell activity of WT and AMPK KO TPC-1 cells processed with PF (0.5 mM) for two days. (B) BrdU assay for cell proliferation of WT and AMPK KO TPC-1 cells cultured in PF for two days. (C) Western blots of indicated proteins within WT or AMPK KO TPC-1 cells treated by PF (0.5 mM) for 48 h. Actin was used for normalization. (D) Transwell migration analysis of WT and AMPK KO TPC-1 cells treated with PF (0.5 mM) for 48 h. (E) The ATP level of WT and AMPK KO TPC-1 cells treated with PF or AICAR for two days, normalized to total cell numbers. (F) The lactate level of WT and AMPK KO TPC-1 cells treated with PF or AICAR for two days, normalized to total cell number. (G) Cell viability of PF-treated TPC-1 cells for two days under normal or glucose restriction (GR) condition. (H) BrdU analysis of cell proliferation of PF-treated TPC-1 cells for 48 h under normal or glucose restriction condition. (I) Transwell migration analysis of TPC-1 cells treated with PF for 48 h under normal or glucose restriction condition. Student's t-tests was used for statistic calculation. NS, $p > 0.05$, $^{*}p < 0.05$; $^{**}p < 0.01$.

Shanghai JiaoTong University, Shanghai, China. Most of the tumor specimens were obtained directly from the surgery. Studies involving patient samples were approved by the Shanghai JiaoTong University review board.

2.2. Cell culture

Human thyroid cancer cell lines BCPAP and TPC-1 were purchased from ATCC. They were cultivated in RPMI 1640 containing 10% fetal bovine serum (FBS) and 1% nonessential amino acids supplemented with glutamine and maintained in a humidified incubator with 5% CO_2 at normal human body temperature. In order to conduct dose-response tests, cells were first grown in RPMI 1640 with 10% FBS, and then RPMI 1640 with less FBS (5%) for 24 h before the addition of various concentrations of drugs, or dimethyl sulfoxide (DMSO) control, in fresh 5% PBS media as previously demonstrated in TC cells. Subsequently, these cells were further cultured for 2 days before being harvested. AMPK activators phenformin (PF) and metformin (MF) were purchased from Sigma. And AICAR was purchased from Selleckchem. Other than described, the concentrations for PF, MF, and AICAR were 0.5 mM, 3 mM, and 0.5 mM respectively. This study has been approved by the Ethics Committee of RenJi hospital, School of medicine, Shanghai JiaoTong University.

2.3. Plasmids, siRNA, and Crispr/Cas9 knockout (KO)

AMPK CRISPR/Cas9 KO plasmid was purchased from Santa Cruz (SC-400104). The generation and screening of AMPK KO cells was based on GFP selection by flow cytometry as previous described [3]. siRNA of PKM2 and β-catenin was synthesized by Shanghai GenePharma, Co., Ltd. The target gene sequences were listed here below: PKM2: CCATAATCGTCCTCACCAA; β-catenin: CATGTGTTGGTAAGCT CTA; control: ATGCTGATCAGTGTCGATT. For AMPK WT and K224A mutant plasmid construction, PCR products were obtained and inserted in the pcDNA3.1 (+) carrier as previous described [24]. The transfection of plasmid and siRNA was conducted with Thermo Scientific lipofectamin 2000 reagent following the producer's instruction.

2.4. Western blot and immunoprecipitation

Cells were lysed for half an hour in ice-cold immunoprecipitation (IP) buffer that contained proteinase inhibiters (Sigma). BCA assessment was utilized to measure sample concentrations, and Western blot assay was used for analysis. For IP, lysed cells were incubated with 5 μg certain antibodies or common rabbit IgG (SC-2027) from Santa Cruz Biotechnology overnight at 4 °C, and then incubated with Protein A/G agarose for 3 h and washed with proteinase inhibiter-mixed buffer. The antibodies used in the Western blot assay included: AMPK (07–350), *p*-AMPK (07–681), Ki-67 (AB9260), Actin (A5441) (Sigma), Viemntin (SC-373717), E-cadherin (SC-71009) (Santa Cruz), PKM2 (#3198) (Cell

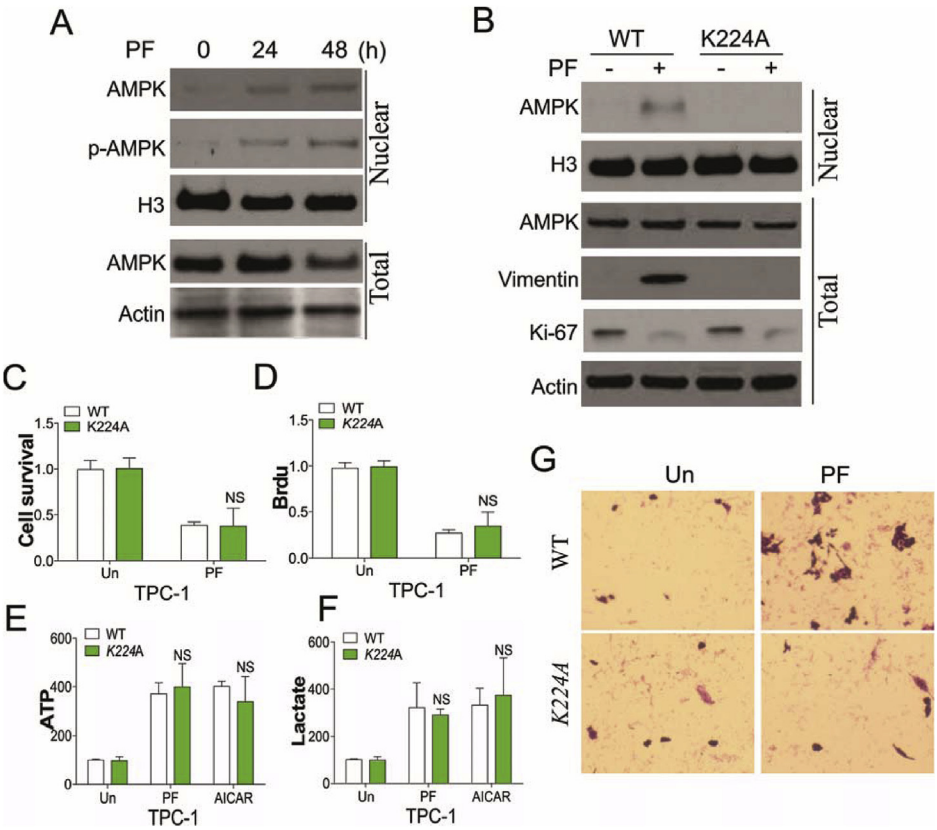

Fig. 3. Nuclear AMPK promotes TC cell migration. (A) The nuclear location of AMPK was analyzed by cellular fractionation followed by Western blot. (B) The AMPK KO TPC-1 cells transfected with WT or K224A mutant AMPK plasmid were treated with PF (0.5 mM) for 48 h. AMPK nuclear translocation and expression of proteins were analyzed through Western blot. (C, D) AMPK KO TPC-1 cells were processed as in (B). The cell viability (C) and cell proliferation (D) were analyzed. (E, F) The AMPK KO TPC-1 cells transfected with WT or K224A mutant AMPK plasmid were treated with PF or AICAR for two days. The ATP (E) and lactate levels (F) were analyzed. (G) The AMPK KO TPC-1 cells were processed similar to that in (B). The cell migration was assessed via transwell migration assay. For the Western blot, H3 expression was used for nuclear fraction normalization, and actin was used for total protein normalization. Student's t-tests was used for statistic calculation. NS, $p > 0.05$, $^{*}p < 0.05$; $^{**}p < 0.01$.

Signaling), H3 (ab18521), β-catenin (ab32572) (Abcam). All of the antibodies were diluted in a 1:1000 for Western blot.

2.5. Cellular fractionation

Cells were lysed with buffer that contained 10 mM each of HEPES and KCl, 1.5 mM $MgCl_2$, 1 mM DTT and 1% Igepal CA-630, followed by centrifugation at the speed of 13200 rpm for 2 min. The supernatant contained cytoplasmic contents. The cell pellets were resuspended with lysis solution, followed by sonication twice with ice before a final centrifugation at 13200 rpm for 10 min. The supernatant contained the nuclear fraction. The cytoplasmic and nuclear fractions were analyzed via Western blot assay.

2.6. Extracted RNA and quantitative RT-PCR

Total RNA was extracted from thyroid carcinoma cells with the RNeasy Mini Kit following the producer's manual. Qiagen's SYBR Green Real-Time qPCR master mix was used. The expression levels of genes that regulated glycolysis were assessed through RT-PCR assays. **MMP7 and c-Myc** expression levels were examined using specific primers: GTATGGGACATTCCTCTGATCC and CCAATGAATGAATGAATGGATG for MMP7; CCTCAACGTTAGCTTCACCAA and TTTGATGAAGGTCTCG TCGTC for c-Myc.

2.7. Pyruvate kinase assessment

Cells were washed in ice-cold PBS and then lysed in pyruvate kinase buffer before harvest. Pyruvate kinase activity was measured via the activity assessment kit (K709) following the manufacturer's instructions. The total protein was adjusted to 3 μg.

2.8. Cell proliferation assays

TC cells were treated with drugs for two whole days, and subsequently pulse-modulated by 5-Bromo-2-deoxyuridine (BrdU) for another 8 h. The proliferation of cells was observed through BrdU incorporated assessment based on the manufacturer's (Roche) directions. The absorbance at 450 nm was detected. Cellular metabolic activity was assessed via 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide (MTT). Afterwards, 50 mg MTT was mixed in the culture medium in every well for two to 4 h till the purple sediment could be observed clearly. The medium was then washed off and an extra 75 μL DMSO was added to each sample, culturing cells without lights at room temperature for 2 h. The absorbance at 490 nm was detected.

2.9. Transwell migration assay

In vitro invasion assays were performed in Matrigel-coated transwells (Thermofisher). TPC-1 cells (5×10^4 in 200 μL serum-free medium) were placed in the top chamber, and the lower chamber was filled with 600 μL of medium with 10% FBS. After 48 h, cells in the upper surface of the transwell membrane with a cotton. Invaded cells on the lower membrane surface were fixed, stained, photographed, and counted.

2.10. ATP generation & glucose uptake assessment

The intracellular ATP was assessed through Bio vision's ATP colorimetric assay kit (K354). The Bioassay system's glucose uptake fluorimetric assay kit (EFGU100) was used to measure uptake levels. Measured figures were normalized to the number of cells and concentration of protein.

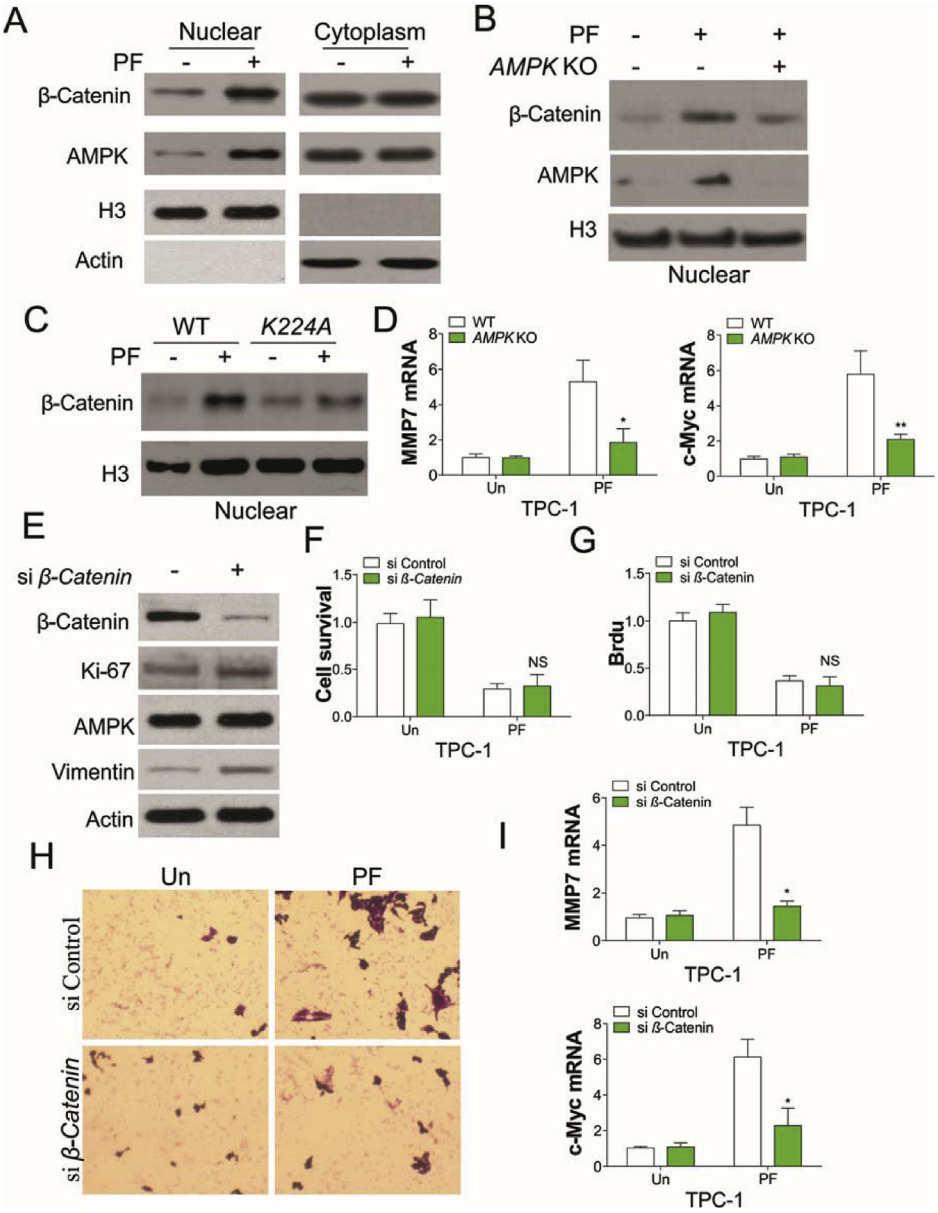

Fig. 4. AMPK promotes TC cell migration by activating β-catenin transcription. (A) The nuclear location of β-catenin within TPC-1 cells upon PF treatment (0.5 mM) for two exact days. (B) The nuclear location of β-catenin in WT AMPK KO TPC-1 cells upon PF treatment for two days. (C) The AMPK KO TPC-1 cells transfected with WT or K224A mutant AMPK plasmid were treated with PF for two days. The nuclear β-catenin was analyzed via Western blot. (D) mRNA levels of MMP7 and c-Myc from WT and AMPK KO TPC-1 cells upon PF treatment for two days. (E) TPC-1 cells that went through transfection with control or β-catenin siRNA were treated with PF for two days. The expression levels of indicated proteins was analyzed via Western blot assay. (F–H) The cell viability (F), proliferation (G) and migration (H) of TPC-1 cells processed as in (E) were analyzed. (I) The mRNA of MMP7 and c-Myc from TPC-1 cells processed as in (E). Student's t-tests was used for statistic calculation. For the Western blot, H3 expression was used for nuclear fraction normalization, and actin was used for total protein normalization. NS, $p > 0.05$, *$p < 0.05$; **$p < 0.01$.

2.11. Statistical methods

To compare statistics, double-grouped non-paired Student's t-tests were carried out by GraphPad Prism. Multiple-group comparisons were performed using one-way analysis of variance (ANOVA)

3. Results

3.1. Activation of AMPK inhibits TC cell growth but promotes its migration

We first investigated the in vitro effect of the AMPK agonists, phenformin and AICAR (at 0.5 mM each), as well as metformin (MF, 3 mM), on thyroid cancer cell proliferation in human TC cells. The metabolic activity of TPC-1 and BCPAP cells that were treated with PF, MF, and AICAR decreased in a time-dependent manner (Fig. 1A). The BrdU assay revealed that these 3 drugs inhibited TC cell proliferation (Fig. 1B). In contrast, AMPK agonists only slightly induced apoptosis in TC cells (Fig. 1C). These results indicated that supplementation with AMPK agonists majorly limited TC cell growth by suppression of proliferation. However, treatment with the AMPK agonists, PF, significantly promoted TC cell migration (Fig. 1D), suggesting that activation of AMPK might increase the TC metastasis. We also analyzed the expression of several markers of cell proliferation (Ki-67) and migration (Viemntin, E-Cadherin), and found that PF treatment led to activation of AMPK, suppression of Ki-67 and E-caderin, and induction of vimentin (Fig. 1E). Furthermore, the expression level of AMPK in TC patients with metastasis is much higher than normal TC patients (Fig. 1F). Therefore, our data indicated that activation of AMPK suppressed TC cell proliferation but enhanced its migration.

3.2. Depletion of AMPK suppressed TC cell migration

To explore the role of AMPK in a thyroid carcinoma cell line, we employed CRISPR/Cas9-technology to knock out (KO) endogenous AMPK in TPC-1 cells. Depletion of AMPK abolished the suppressive effect of PF on TC cell proliferation (Fig. 2A–C). In addition, AMPK KO also suppressed the TC cell migration induced by PF (Fig. 2C and D), suggesting that AMPK plays dual roles in TC cell proliferation and migration. AMPK plays an important role in glycolysis [26], we therefore tested whether the functions of AMPK were regulated by glucose

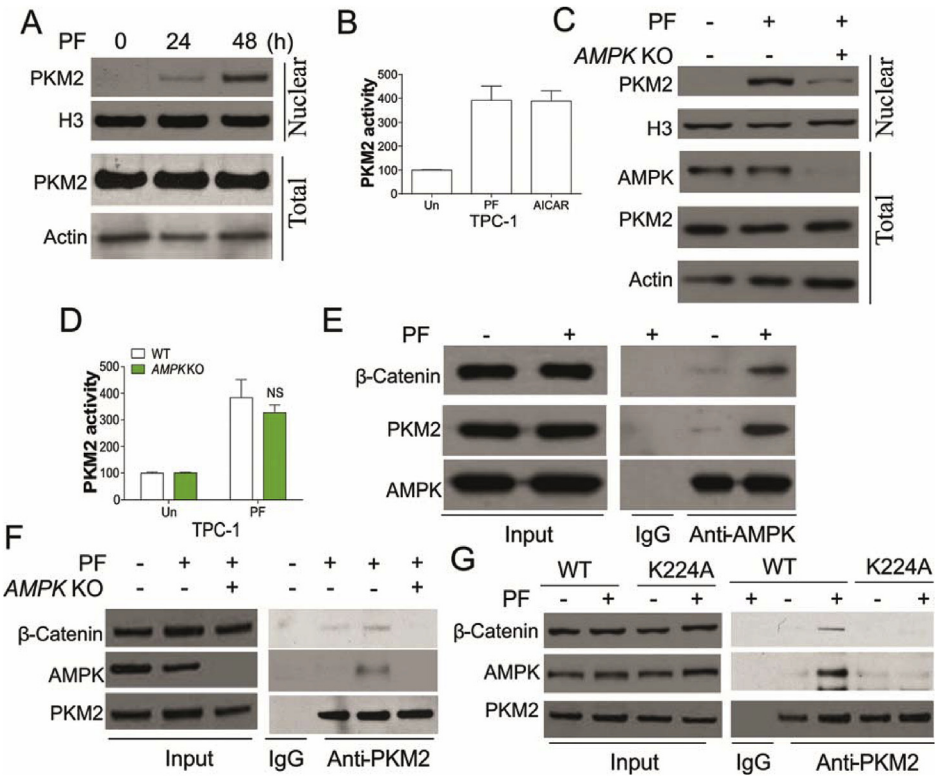

Fig. 5. AMPK interacts with PKM2 and β-catenin in the nucleus. (A) The nuclear location of PKM2 within TPC-1 cells upon treatment with PF (0.5 mM) for 48 h. (B) The PKM2 activity in WT TPC-1 cells treated with PF for 48 h. (C) The nuclear location of PKM2 in WT and AMPK KO TPC-1 cells upon PF treatment for 48 h. (D) The PKM2 activity in WT and AMPK KO TPC-1 cells treated with PF for 48 h. (E) The interaction of AMPK, β-catenin, and PKM2 was assessed via immunoprecipitation. (F) The interaction between β-catenin and PKM2 in WT and AMPK KO TPC-1 cell was analyzed via immunoprecipitation. (G) The AMPK KO TPC-1 cells transfected with WT or K224A mutant AMPK plasmid were treated with PF for two days. The interactions between PKM2 and β-catenin were analyzed. For the Western blot, H3 expression was used for nuclear fraction normalization, and actin was used for total protein normalization. Student's t-tests was used for statistic calculation. NS, $p > 0.05$.

metabolism. Supplementation with PF or AICAR increased the ATP and lactate levels in TPC-1 cells, which was eliminated by AMPK depletion (Fig. 2E and F), suggesting that glycolysis might contribute to AMPK effects on cell proliferation and migration. To further confirm this hypothesis, we cultured the TPC-1 cells in a glucose restriction (GR) condition. Under GR, treatment with PF demonstrated more inhibitory effects on TPC-1 cell proliferation (Fig. 2G and H). However, GR treatment did not change the TPC-1 migration induced by PF (Fig. 2I). These results collectively suggested that activation of AMPK mediated TPC-1 migration independent of its effect on glycolysis.

3.3. Nuclear AMPK is important for TC cell migration

AMPK is a critical nutrient factor, and previous research has shown that it could transfer to the nucleus upon energy constraint stress [29]. Our research indicated that PF treatment actually induced AMPK activation through up-regulating its phosphorylation and inducing its nuclear transition (Fig. 3A). To further confirm the effect of nuclear transition of AMPK, AMPK KO TCP-1 cells were transfected with plasmids carrying WT AMPK or a nuclear translocation defective AMPK (K224A). As predicted, mutation of AMPK did not translocate into the nucleus when compared with the WT AMPK transfection (Fig. 3B). Re-expression of WT or mutant AMPK suppressed the expression of Ki-67 (Fig. 3B), and proliferation of AMPK KO TPC-1 cells (Fig. 3C and D), and resumed the production of ATP and lactate (Fig. 3E and F). However, rescue of WT AMPK expression also promoted TPC-1 cell migration, which is not observed in mutant AMPK (K224A) expressing TPC-1 cells (Fig. 3B, G). Therefore, it might be that GR increases nuclear translocation of PKM2 and AMPK through AMPK activation.

3.4. AMPK mediates TC cell migration by recruiting β-catenin into the nucleus

AMPK was reported to promote β-catenin nuclear translocation, which mediates cancer cell migration [6,16,31]. We therefore tested whether nuclear transfer of β-catenin is coupled with a change in location of AMPK. As predicted, PF treatment in TPC-1 cells promoted β-catenin nuclear translocation (Fig. 4A). Depletion of AMPK abolished β-catenin nuclear transition (Fig. 4B). Re-expression of WT AMPK resumed the β-catenin nuclear transition, which was not seen with the re-expression of mutant AMPK (Fig. 4C). Furthermore, PF treatment enhanced the expression levels of β-catenin downstream targets, such as MMP7 and c-Myc, which are correlated with cell migration [6] (Fig. 4D). Depletion of AMPK suppressed the mRNA levels of these two genes (Fig. 4D), further suggesting that AMPK mediated the transcriptional activity of β-catenin. To fully understand the function of β-catenin on AMPK activation-mediated cell migration, siRNA was utilized to knockdown β-catenin upon PF treatment within TPC-1 cells. Silence of β-catenin in TPC-1 cells treated with PF did not affect the activation of AMPK and suppression of Ki-67, but suppressed the induction of vimentin, which is a migration marker (Fig. 4E). Accordingly, depletion of β-catenin did not have any effects on cell proliferation inhibition caused by PF treatment in TPC-1 cells (Fig. 4F and G), but suppressed the migration induced by PF (Fig. 4H), as well as the mRNA levels of MMP7 and c-Myc (Fig. 4I). Collectively, our results suggested that activation of AMPK promotes TC cell migration by recruiting β-catenin into the nucleus.

3.5. AMPK promotes β-catenin nuclear translocation by binding with PKM2

Next, we were interested in understanding the underlying mechanism for β-catenin nuclear translocation by AMPK. It was reported that activated AMPK interacts with PKM2 [29], which can promote the transcription of β-catenin and trigger migration of cells [27]. Our results revealed that PF escalated the nuclear translocation of PKM2 in TPC-1 cells (Fig. 5A). Activated AMPK by PF/AICAR enhanced the PKM2 activity as well (Fig. 5B), which is positively correlated with glycolysis (Fig. 2B). Depletion of AMPK suppressed the nuclear translocation of PKM2 (Fig. 5C), but did not affect its kinase activity (Fig. 5D), suggesting that the activity of PKM2 may be irrelevant to the AMPK-triggered TC cell migration. We further studied the relation between AMPK, β-catenin, and PKM2, and found that PF treatment

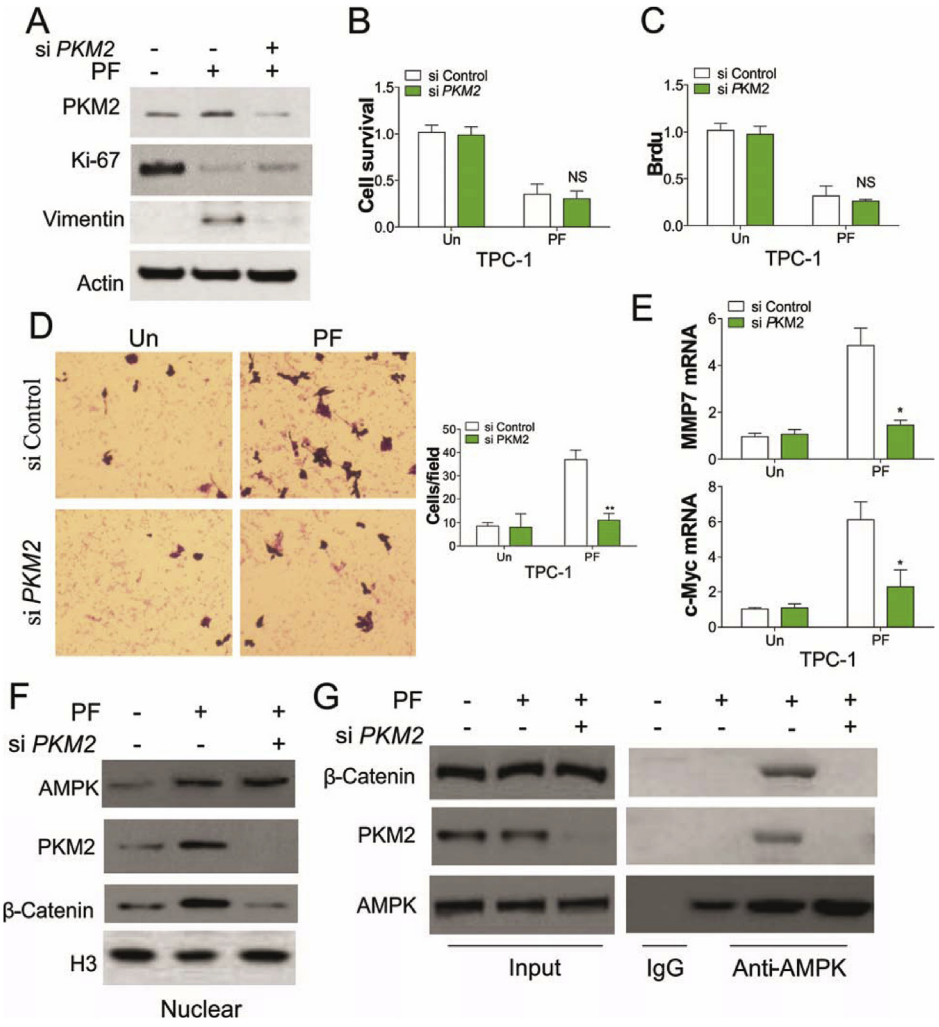

Fig. 6. PKM2 mediated the interaction of AMPK and β-catenin. (A) TPC-1 cells that were transfected with control or PKM2 siRNA were treated with PF (0.5 mM) for two days. The expression levels of the indicated proteins were analyzed by Western blot. (B–D) The cell viability (B), multiplication (C), and migration (D) of TPC-1 cells treated as in (A) were analyzed. (E) The mRNA levels of MMP7 and c-Myc in TPC-1 cells processed like in (A). (F) The nuclear location of AMPK and β-catenin in TPC-1 cells processed like in (A). (G) The interaction of AMPK, PKM2 and β-catenin within TPC-1 cells treated as in (A) were analyzed by immunoprecipitation using anti-AMPK. For the Western blot, H3 expression was used for nuclear fraction normalization, and actin was used for total protein normalization. Student's t-tests was used for statistic calculation. NS, $p > 0.05$, *$p < 0.05$; **$p < 0.01$.

enhanced the interaction of three proteins (Fig. 5E). Depletion of AMPK abolished the interaction of β-catenin and PKM2 (Fig. 5F). The interaction of AMPK with PKM2 and β-catenin was dependent on the nuclear translocation of AMPK, since the expression of mutant AMPK did not interact with PKM2 or β-catenin (Fig. 5G). Therefore, our results suggested that AMPK promotes β-catenin nuclear translocation and its interaction with PKM2.

3.6. PKM2 is necessary for AMPK mediated TC cell migration

To better understand the function of PKM2 in AMPK-modulated TC cells migration, we depleted PKM2 by siRNA transfection. Absence of PKM2 in TPC-1 cells did not affect the inhibitory effects of PF on cell proliferation (Fig. 6A, B, C). In contrast, PKM2 depletion abrogated the TC cell migration induced by PF treatment in TPC-1 cells (Fig. 6A, D). Accordingly, PKM2 knockdown also suppressed the induction of MMP7 and c-Myc mRNA levels (Fig. 6E), suggesting the inhibition of β-catenin transcription activity. Absence of PKM2 did not affect the nuclear translocation of AMPK, but suppressed nuclear accumulation progression of β-catenin (Fig. 6F), and dissociated the interaction of AMPK and β-catenin (Fig. 6G). Therefore, our results suggested that PKM2 is necessary for AMPK recruitment β-catenin into the nucleus, and promotes transcription of several genes related to cell migration.

4. Discussion

AMPK has always been viewed to be able to restrain tumorous progression, and former studies mainly concentrated on exploring the impact of AMPK on the suppression of cell growth. The AMPK activators PF, MF, and AICAR, have been utilized to prevent and treat cancers. Nevertheless, there was still a major dispute that related to the role of AMPK in tumor formation and cancerous development, since recent research suggests that AMPK activation showed no connection with the reduced danger of lung, rectal, or breast cancers [29]. Nevertheless, the AMPK pathway has not been comprehensively researched in the context of thyroid carcinoma and the expression levels and function of AMPK in the above-mentioned disease had not been examined up to now. Our data demonstrated that although activated AMPK suppresses the proliferation of TC cells, it also promotes TC cell migration, which might lead to unfavorable clinical outcomes for TC patients. We found that after treatment with AMPK stimulator not only enhances its kinase activity, but also lead to its nuclear translocation. The nuclear AMPK then recruits PKM2 and β-catenin into the nucleus, which activates the transcription of β-catenin downstream genes. Our study described a novel function of AMPK in the cancer metastasis, and provided a new strategy to overcome this drawback.

AMPK has long been categorized as a primary regulator of cell metabolism, and during the process activated AMPK activates a general rise in catabolic processes [14]. Different methods have been recently utilized to regulate AMPK activities among cancers. Choi et al. recorded that treatment with AICAR could trigger a decline of cell generation and stimulate apoptosis in thyroid carcinoma cell lines, showing wild-type or V600E-mutant BRAF [7]. The anti-proliferous function of metformin among cancerous cells has great reliance on the concentration of

glucose in the milieu outside cells [1]. Echoing these former findings about the anti-proliferative effect of AMPK, we further proved that activated AMPK that was induced by PF, MF, or AICAR inhibited TC cell proliferation, but with seldom apoptotic signals. However, different researchers have shown that AMPK activation that was regulated via reducing ATP/AMP rate enhanced cell survivability in stressed-out metabolic situations within the tumor microenvironment [2,17]. Moreover, antagonists of AMPK like compound C suppress cell generation and migration in prostatic carcinoma [10,13]. Therefore, it is unclear if agonists or antagonists should be applied with respect to the specific function of AMPK in different cases (oncogenic vs. tumor inhibitive). In our study, we firstly clarified the effect of AMPK on TC cell proliferation and metastasis. Although AMPK activation processes the tumor suppression function, it also enhances TC cell migration. Different from early findings that AMPK was a nutrient detector within the cytoplasm, this research revealed that the effect of AMPK was independent of cell metabolism, since glucose deprivation did not affect the cell migration induced by AMPK activation. Instead, the nuclear translocation of AMPK mediated the TC cell migration, which reveals a new capability of AMPK. The findings of this study suggest a novel function for AMPK in considerably enhancing cancerous cell migration attributes upon activation that relates to a new nuclear transition pathway for PKM2. Our study revealed the underlying relationship between PKM2, AMPK, and β-catenin, and displayed the mediating function of proteins upon PKM2 and β-catenin nuclear accumulation. It was also found that depletion or inhibition of PKM2 can suppress the TC cell migration induced by AMPK activation. Thus, orchestrating the signaling pathways of AMPK and PKM2 is important for the interplay between tumor progression and metastatic spread, and will benefit TC patient therapy.

PKM2 is the primary protein isoform of pyruvate kinase for activation of leukemia. PKM2 is observed to have great influence on the metabolic activities of cancerous cells, since inhibition of PKM2 by peptide aptamers suppressed cell development [23] and PKM2 reduction mediated by RNAi or by PKM2 displacement by PKM1 [8] considerably restricted tumor formation in mice. Since PKM2 is an essential element for aerobic glycolysis of cancerous cells, it serves as a read-out for cancer metabolism and is the key power source of cancer cell generation and survival [8]. Though the kinase activity of PKM2 closely relates to the regulation of aerobic glycolysis among cancer cells, PKM2 transition to the nucleus also has a critical role in the expression levels of c-Myc, a β-catenin target gene [27,28]. These discoveries indicate that the nucleus is the central position for PKM2 in terms of tumor generation and the Warburg effect. Moreover, nuclear PKM2 regulates the epithelial–mesenchymal transition (EMT) of cancerous cells with activated EGF and TGF-β, leading to the inhibition of E-cadherin transcription [28]. This research demonstrated that PKM2 is transferred to the nucleus with AMPK activation in TC cells, and necessary for their interaction with β-catenin, which is consistent with most recent study about the complex of PKM2, AMPK and β-catenin formation [16]. The above outcomes indicate that the nuclear transition of PKM2 was the reason for cancer cell migration upon AMPK activation. It has been demonstrated that nuclear PKM2 expression correlates with cancer grading and poor prognosis of patients with glioma and esophageal squamous cell carcinoma [27]. Therefore, targeting the nuclear transition of PKM2 in combination with AMPK activating drugs could well become a new pathway to treat TC cancer.

5. Conclusions

In general, our results revealed a dual role of AMPK in mediating TC cell proliferation and migration. The important function of AMPK in multiple physiological activities may finally rule AMPK out from being a possible therapeutic target because any changes in it will exert various negative consequences. Hence, future TC cancer therapy could target the suppression of nuclear PKM2 and relative complexes to weaken the side effects caused by AMPK activation.

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Declaration of competing interest

The authors declare no conflict of interest.

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