Acta Pharmacologica Sinica 2017: 1401–1411
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www.nature.com/aps

Original Article

Blocking the utilization of glucose induces the switch from senescence to apoptosis in pseudolaric acid B-treated human lung cancer cells *in vitro*

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Abstract

Pseudolaric acid B (PAB), a diterpene acid isolated from the root bark of *Pseudolarix kaempferi* Gordon, exerts anti-tumor effects in several cancer cell lines. Our previous study showed that PAB mainly induced senescence via p53-p21 activation rather than apoptosis in suppression of the growth of human lung cancer A549 cells (p53 wild-type). In p53-null human lung cancer H1299 cells, however, PAB caused apoptosis without senescence. In this study we investigated what mechanism was responsible for the switch from senescence to apoptosis in PAB-treated human lung cancer cell lines. Senescent cells were examined by SA-β-gal staining. Glucose uptake and the apoptosis ratio were assessed using a FACScan flow cytometer. Commercial assay kits were used to measure the levels of ATP and lactate. Transfection of siRNA was used to knockdown the expression of p53 or p21. Western blot analysis was applied to measure the protein expression levels. In p53 wild-type A549 cells, PAB (20 μmol/L) caused senescence, and time-dependently increased glucose utilization; knockdown of p53 or p21 significantly decreased the uptake and metabolism of glucose but elevated PAB-induced apoptosis. Inhibition of glucose utilization using a glycolytic inhibitor 2-DG (1 mmol/L) significantly enhanced apoptosis induction. Similar results were observed in another p53 wild-type H460 cells treated with PAB. Opposite results were found in p53-null H1299 cells, where PAB time-dependently decreased glucose utilization, and induced only apoptosis. Our results demonstrate that PAB-induced senescence is associated with enhanced glucose utilization, and lower glucose utilization might contribute to apoptosis induction. Thus, blocking glucose utilization contributes to the switch from senescence to apoptosis, and p53 plays an important role in this process.

Keywords: pseudolaric acid B; human lung cancer; senescence; apoptosis; glucose utilization; p53

Acta Pharmacologica Sinica (2017) 38: 1401–1411; doi: 10.1038/aps.2017.39; published online 26 Jun 2017

Introduction

Many cancer cells consume glucose avidly and produce lactic acid rather than catabolizing glucose via the tricarboxylic acid cycle (TCA) cycle. Cancer cells preferentially utilize glycolysis instead of oxidative phosphorylation in the presence of oxygen, despite an enormous demand for ATP to supply their growth. Glycolysis is much better suited to meeting the demand for the survival of cancer cells[1,2]. The advantage of the glycolytic metabolism is the production of glycolytic intermediates that can be used in anabolic pathways, allowing for the synthesis of the proteins, nucleic acids and lipids required for proliferation[3]. Therefore, the poor energetic pay-off of glycolysis by a high glucose supply is compensated.

This aerobic glycolysis is known as the “Warburg effect” and is considered a hallmark of cancer[4,5]. The transmembrane glucose transporter (Glut) protein mediates glucose uptake, which is required for the rapid proliferation of cancer cells[6]. Hexokinase is the first and rate-limiting enzyme during glycolysis and catalyzes the conversion of glucose to glucose-6-phosphate (G-6-P)[7]. The production of ATP or lactate by glycolysis might be an advantage for tumor survival and growth[7,8]. Most tumor cells primarily utilize glucose via aerobic glycolysis for their energy needs, suggesting that targeting the metabolic changes could be an effective strategy for cancer treatment[9].

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Received 2016-12-19 Accepted 2017-03-23

Pseudolaric acid B (PAB) exerts multiple biological and pharmacological activities, including anti-fungal, -tumor, -fertility and -microtubule properties[10]. Relationships between the molecular mechanisms of apoptosis and G_2/M cell cycle arrest induced by PAB have been extensively studied[11-13]. However, our previous studies demonstrated that PAB partially induced senescence in human breast cancer MCF-7 and murine fibrosarcoma L929 cells[11,14]. In addition, PAB mainly induced senescence rather than apoptosis in p53 wild-type A549 cells[15]. However, PAB caused apoptosis without senescence in p53-null H1299 cells[15].

Although the induction of cellular senescence is a well-established therapeutic strategy for tumor suppression, adverse actions are reported in some cases; for example, the secretion of tumor cell growth-promoting factors has been reported to cause chemoresistance[16,17]. Apoptosis is still one of the most effective anti-cancer strategies in cancer therapy, but the side effects on normal cells remain an important issue[18]. Understanding the advantages and drawbacks between senescence and apoptosis might provide a novel strategy for cancer therapy.

Here, we apply p53 wild-type and null cell lines to investigate the relationship between senescence and apoptosis induced by PAB. Additionally, the role of glucose utilization is also evaluated.

Materials and methods

Reagents

PAB with 99% purity was obtained from the National Institutes for Food and Drug Control (Beijing, China). PAB was dissolved in dimethylsulfoxide (DMSO) as a stock solution. The concentration of DMSO was maintained below 0.1% in all the cell cultures to prevent any detectable effect on cell growth. 2-Deoxy-*D*-glucose (2-DG) was purchased from Sigma Chemical Co (St Louis, MO, USA). Primary antibodies against p-p53 (Ser 15), p53, p21, AMPK, p-AMPK (Thr 172), caspase 3 and β-actin as well as horseradish peroxidase-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Primary antibodies against Glut-1 and hexokinase-2 (HK-2) were purchased from Proteintech Group (Chicago, IL, USA). The SuperSignal® West Pico Chemiluminescent Substrate for horseradish peroxidase (HRP) was obtained from Thermo Scientific (Rockford, IL, USA).

Cell culture

A549, H460 and H1299 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). A549 cells were cultured in DMEM (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin. H460 and H1299 cells were cultured in RPMI-1640 (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were incubated at 37°C with 5% CO_2 in a humidified atmosphere. Logarithmically growing cells were used in all the experiments.

Cell viability assay

To determine the effect of glycolysis inhibition on cell viability, the cells were treated with or without 2-DG at the given concentrations for 1 h and were subsequently treated with PAB for the indicated time periods. The effect was measured by the MTT assay. The percentage of cell viability was calculated as follows:

$$\text{Cell viability (\%)}=(A_{492,\text{sample}}-A_{492,\text{blank}})/(A_{492,\text{control}}-A_{492,\text{blank}})\times 100$$

SA-β-Gal staining

To detect senescent cells, the cellular senescence assay kit (Beyotime, Shanghai, China) was used according to the manufacturer's instructions. The cells were washed twice with PBS and were incubated in 1 mL of fixing solution at room temperature for 15 min. After removing the fixing solution followed by washing the fixed cells three times with PBS, the cells were stained with 1 mL of the working solution for freshly prepared cell staining at 37 °C for at least 12 h under protection from light. After staining, the cells were washed, and senescent cells were identified as blue-stained cells by light microscope (Olympus, Tokyo, Japan). At least 200 cells per field of vision were counted for each sample in three random fields to determine the percentage of SA-β-Gal-positive cells.

Apoptosis detection

Quantitative analysis of apoptotic cell death caused by PAB with or without 2-DG was analyzed using the Annexin V-FITC Apoptosis Detection Kit (KeyGEN BioTECH, Nanjing, Jiangsu, China) following the manufacturer's protocol. The cells were analyzed with a FACScan flow cytometer.

Glucose uptake assay

The cells were seeded and treated with drugs for the indicated period of time. Subsequently, the cells were washed twice with PBS and were incubated with 10 μmol/L of the fluorescent glucose analog 2-(N-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino)-2-deoxyglucose (2-NBDG; Invitrogen, Carlsbad, CA, USA)) in the dark at 37 °C for 2 h. The cells were collected, and glucose uptake was assessed by FACScan flow cytometry (Becton Dickinson, Franklin Lakes, NJ, USA).

Intracellular ATP assay

The intracellular ATP concentration was measured using the ATP Assay Kit (Beyotime, Shanghai, China). Briefly, cells were cultured in DMEM or 1640 medium for 24 h, followed by subsequent experiments. The ATP levels were measured according to the manufacturer's instructions. The ATP levels were expressed in terms of the protein content for signal normalization.

Lactate production assay

The cells were seeded into 6-well cell culture clusters (Corning, NY, USA) at a density of 1.5×10^4. After 24 h of incubation, the cells were treated with various drugs. For the assessment

of lactate production, the media were collected and estimated using the Lactate Assay Kit (Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) according to the manufacturer's instructions. The cells were counted and used to normalize the relative lactate levels.

Western blot analysis

Cells were harvested, washed twice with PBS, and then were lysed in whole-cell RIPA lysis buffer (Beyotime, Shanghai, China) supplemented with PMSF (1 mmol/L). After 15 000×*g* centrifugation at 4°C for 15 min, the protein concentration was determined using the Bio-Rad protein assay reagent (Bio-Rad, Hercules, CA, USA). After denaturation with boiling water for 5 min, the lysates containing the same amount of protein were separated by 12% SDS-PAGE and were transferred onto a Millipore Immobilon®-P Transfer Membrane (Millipore, Billerica, MA, USA). The membranes were soaked in 5% skim milk, and then they were incubated with primary polyclonal antibodies overnight, followed by incubation with the corresponding HRP-conjugated secondary antibodies. The blots were visualized using the SuperSignal® West Pico Chemiluminescent Substrate.

Transfection of siRNA

Human p53-targeted (p53-siRNA), p21-targeted (p21-siRNA) and negative control (control-siRNA) siRNAs were purchased from GenePharma (Suzhou, Jiangsu, China). The cells were transfected with 5 nmol/L p53-, p21- or control-siRNA using siRNA-Mate™ (GenePharma, Suzhou, Jiangsu, China) according to the manufacturer's protocols. The transfected cells were used 24 h later for subsequent experiments.

Statistical analysis

The data from at least three independent experiments were presented as the mean±SD. Statistical comparisons were analyzed by one-way ANOVA using Statistics Package for Social Science software (version 13.0; SPSS, Chicago, IL, USA), and LSD-*post hoc* test was employed to assess the statistical significance of the difference between the control and treated groups. $P<0.05$ were considered to be statistically significant.

Results

PAB causes senescence and increases the utilization of glucose in A549 cells

In our previous study, we demonstrated that PAB primarily caused senescence rather than apoptosis in A549 cells[15]. To determine glucose utilization in senescence-capable cells after PAB treatment, flow cytometry was applied to detect glucose uptake. The results showed that PAB induced senescence accompanied by the increase in glucose uptake (Figure 1A, 1B). Next, we assessed whether PAB showed any effect on glucose transport in A549 cells. Western blot analysis indicated that PAB increased the expression of glucose transport protein 1 (Glut-1), and hexokinase 2 (HK-2), a rate-limiting enzyme during glycolysis, was enhanced by PAB treatment (Figure 1E, 1F). Further study also showed that PAB increased the level of ATP (Figure 1C) and lactate production (Figure 1D). Moreover, PAB enhanced the activation of AMPK (adenosine mono-phosphate (AMP)-activated protein kinase), a central energy sensor of the cell, suggesting that senescence-capable cells demanded much more bioenergy (Figure 1E, 1F). This result was consistent with a higher ATP level in PAB-induced senescent cells (Figure 1C). Collectively, these results exploited that PAB increased senescence-capable cells and enhanced the utilization of glucose.

P53 inactivation decreases PAB-induced high glucose utilization in A549 cells

Since p53 inhibition switched PAB-induced senescence to apoptosis[15], the role of p53 in the regulation of glucose utilization was studied in subsequent experiments. Flow cytometric assay showed that the knockdown of p53 or p21 decreased the glucose uptake in PAB-treated A549 cells (Figure 2A). Accordingly, the ATP level and lactate production were attenuated by p53 or p21 inhibition in senescent A549 cells with PAB treatment (Figure 2B, 2C). Inactivation of p53 or p21 decreased the expression of senescent proteins but increased the cleavage of caspase 3 (a classical apoptotic protein) (Figure 2D, 2E). At the same time, the levels of glucose transport protein, a rate-limiting glycolytic enzyme, and phosphorylated AMPK were all inhibited by p53 or p21 interference in PAB-treated A549 cells (Figure 2D, 2E). These results demonstrated that p53 and p21 activation played important roles in PAB-induced higher utilization of glucose in A549 cells.

Inhibition of glucose utilization enhances apoptosis induction in PAB-treated A549 cells

The present study showed that the downregulation of senescence by p53-p21 inhibition decreases glucose utilization. Meanwhile, apoptosis induction is enhanced. Subsequently, a glycolytic inhibitor 2-deoxy-*D*-glucose (2-DG), targeting hexokinase, which is the entry-point enzyme for glycolysis, was applied to further investigate the relationships among glucose utilization, senescence and apoptosis. The MTT assay showed that PAB attenuated cell viability in the presence of 2-DG in A549 cells (Figure 3A). Consistently, 2-DG deceased senescence-capable cells but enhanced the apoptotic ratio (Figure 3B, 3C, 3G, 3H) in PAB-treated A549 cells. More generally, 2-DG attenuated the increased glucose utilization by treatment with PAB (Figure 3D-3F). These results indicated that senescence induction was accompanied by higher glucose utilization; however, apoptotic cells were elevated when blocking glucose metabolism.

Glucose utilization regulates the switch between senescence and apoptosis

To investigate whether the regulation of glucose utilization in senescence and apoptosis was specific for A549 cells, we further assessed the effect of PAB on H460 cells, which is another p53 wild-type cell line. The glucose uptake assay suggested that PAB might enhance the utilization of glucose in H460 cells (Figure 4A). Similar results to those of A549 cells

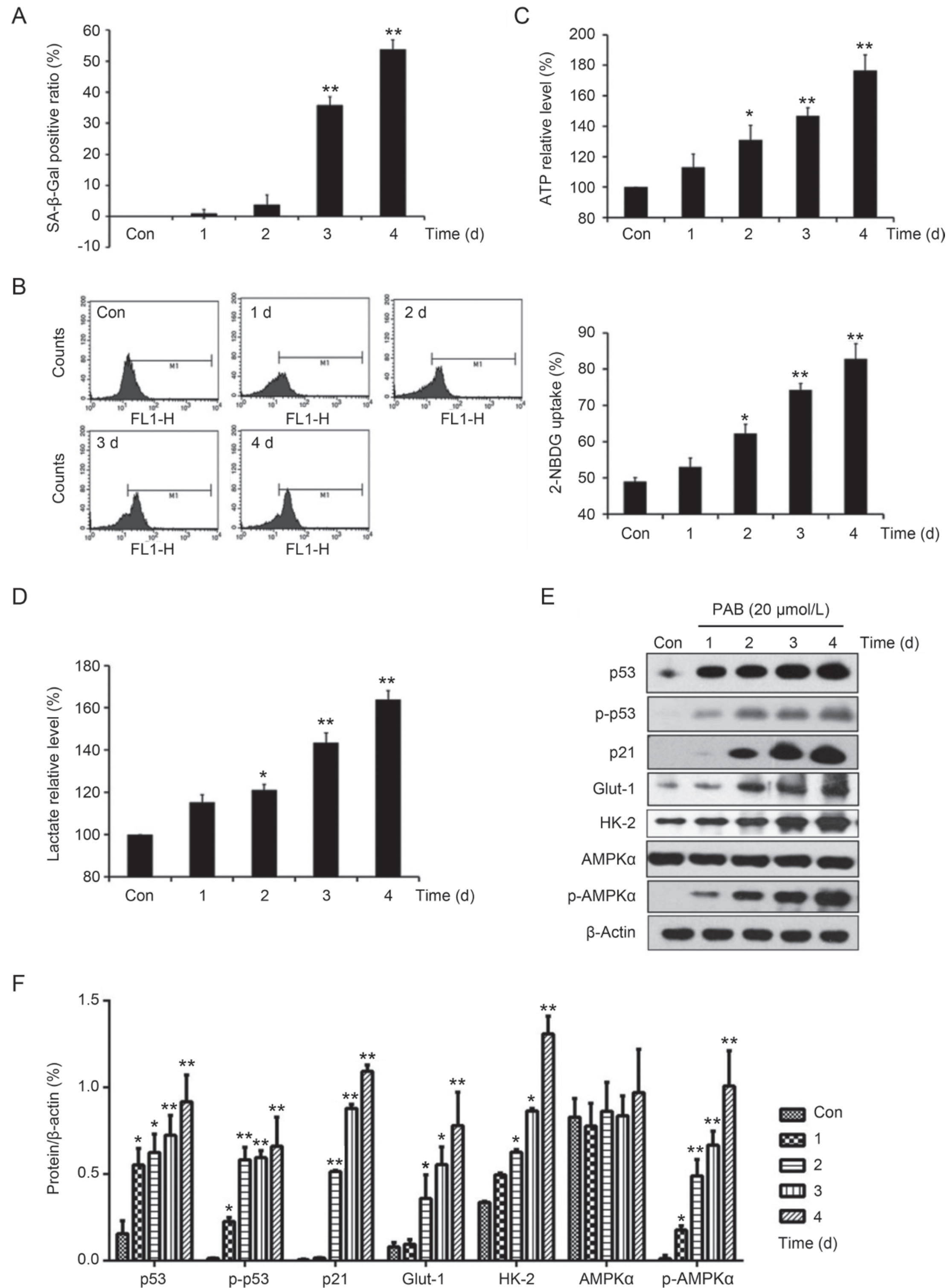

Figure 1. PAB caused senescence and increased the utilization of glucose in A549 cells. The cells were treated with 20 μmol/L PAB for 1, 2, 3 and 4 d. (A) Senescent cells were examined by SA-β-Gal staining, and the ratio of the SA-β-Gal-positive cells is shown. (B) Glucose uptake was determined by flow cytometric analysis with 2-NBDG staining. The right panel indicated the ratio of glucose uptake. (C) The intracellular relative ATP level was detected using a commercial ATP assay kit. (D) The production of lactate was examined using the lactate assay kit. (E) The protein levels of p53, p-p53 (Ser15), p21, Glut-1, HK-2, AMPKα and p-AMPKα (Thr172) were evaluated by Western blotting. (F) ImageJ software was applied to quantify the band densities of proteins. The data are presented as mean±SD of three independent experiments. *$P<0.05$, **$P<0.01$ *vs* the control group.

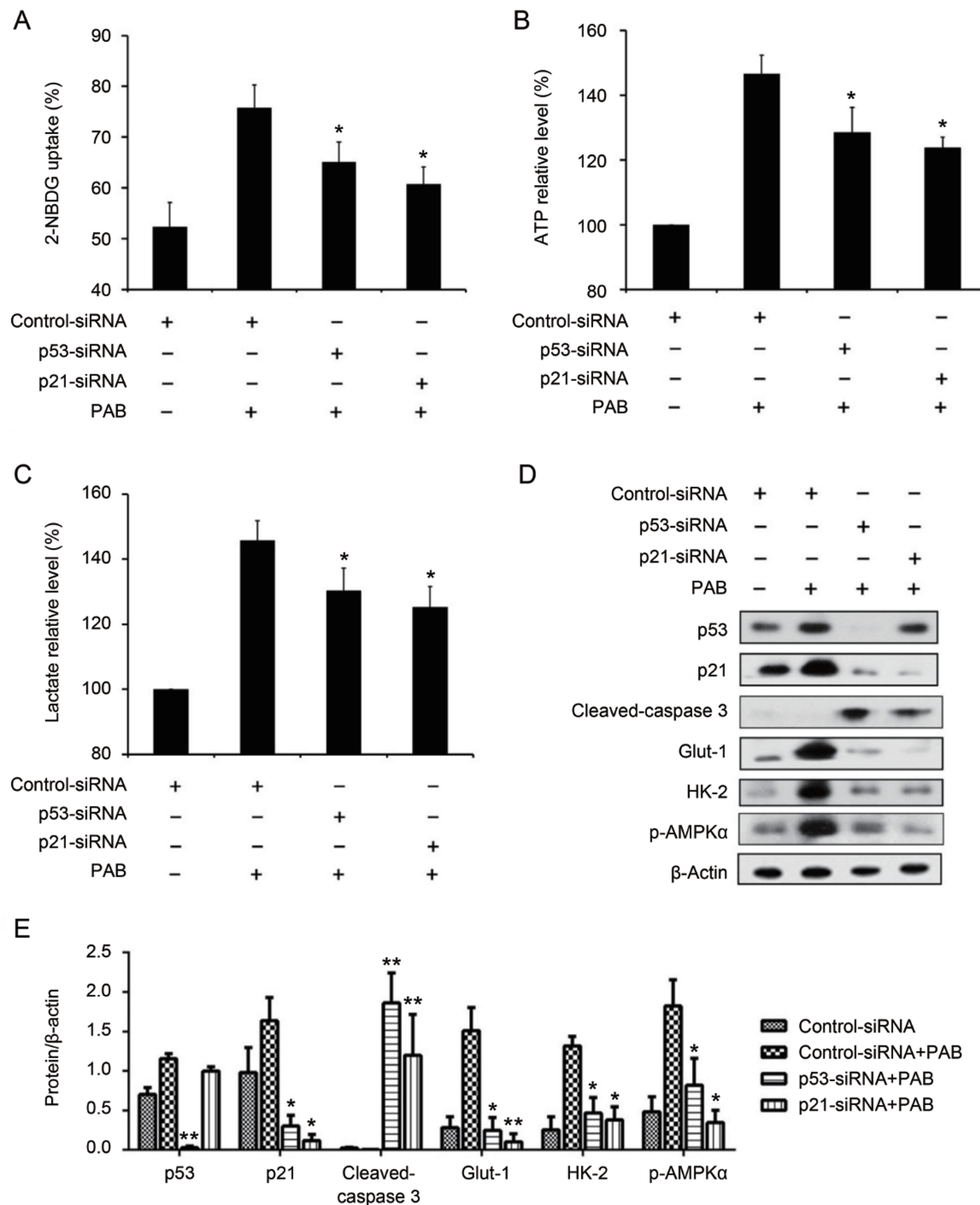

Figure 2. P53 inactivation decreased PAB-induced high-glucose utilization in A549 cells. A549 cells were transfected with control, p53 or p21 siRNA for 24 h. (A) The cells were stained with 2-NBDG and were measured by flow cytometry. The ratio of glucose uptake was calculated. (B, C) Commercial kits were applied to examine the relative level of ATP (B) and lactate (C), respectively. (D) Western blotting was used to determine the levels of p53, p21, cleaved caspase 3, Glut-1, HK-2 and p-AMPKα (Thr172). (E) ImageJ software was used to calculate the band densities of proteins. The data are presented as the mean±SD of three independent experiments. $^{*}P<0.05$, $^{**}P<0.01$ *vs* the PAB-treated group.

regarding the ATP and lactate levels were shown following PAB treatment (Figure 4B, 4C). PAB caused senescence rather than apoptosis in H460 cells (Figure 4D, 4E). Meanwhile, the expression levels of Glut-1, HK-2 and p-AMPK were elevated in PAB-treated H460 cells (Figure 4D, 4E). Knockdown of p53 and p21 also decreased glucose utilization and increased apoptosis induction (Figure 4F, 4G). Subsequently, PAB-induced senescence was switched to apoptosis accompanied by poorer cell viability after treatment with a glycolytic inhibitor (Figure 4H-4L). These results indicated that glucose utilization regulated apoptosis, and senescence might require functional p53.

To further evaluate the role of p53 in this effect, we investigated whether H1299 cells (p53-null) had an opposite result because PAB induced only apoptosis in this cell line[15]. As expected, PAB decreased glucose uptake, and the levels of ATP and lactate were also attenuated in H1299 cells (Figure 5A-5C). The protein expression levels of glucose and energy metabolism were downregulated in PAB-treated H1299 cells, while the level of cleaved caspase 3 was elevated (Figure 5D, 5E). These results demonstrated that PAB-induced senescence

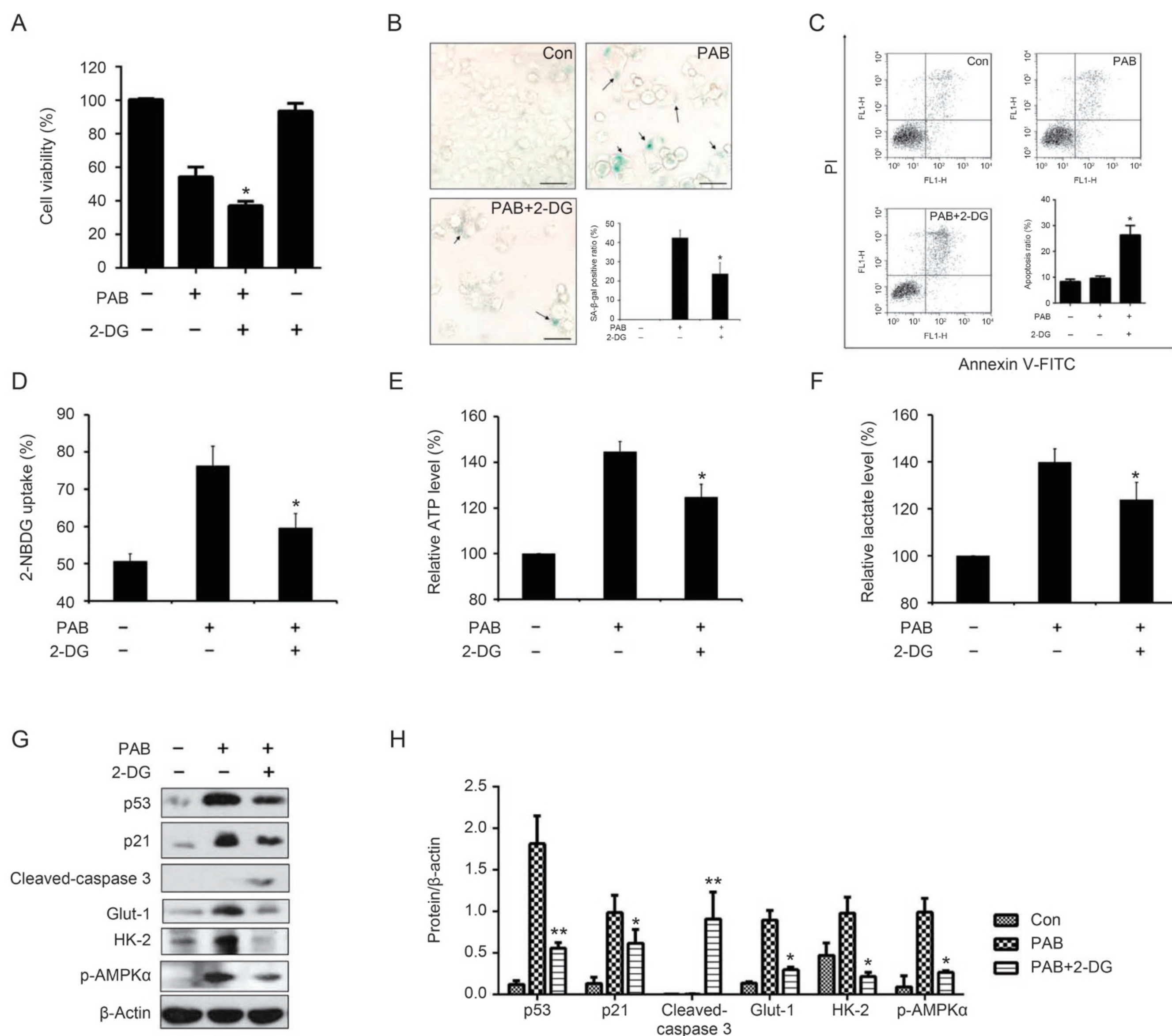

Figure 3. Inhibition of glucose utilization enhanced apoptosis induction in PAB-treated A549 cells. Cells were incubated with 20 μmol/L PAB or combined with 1 mmol/L 2-DG for 3 d. (A) The MTT assay was used to determine the cell viability. (B) SA-β-Gal staining was used to examine the percentage of senescent cells. The ratio of the SA-β-Gal-positive cells was indicated. (C, D) Flow cytometric analysis was applied to determine the ratio of apoptosis with Annexin V-FITC/PI staining and glucose uptake with 2-NBDG staining, respectively. (E, F) ATP and lactate levels were measured using commercial assay kits. (G) Western blotting was utilized to examine the expression of p53, p21, caspase 3, Glut-1, HK-2 and p-AMPKα (Thr172). (H) ImageJ software was used to quantify the band densities of proteins. The data are presented as the mean±SD of three independent experiments. $^{*}P<0.05$, $^{**}P<0.01$ *vs* the PAB-treated group.

was associated with enhanced glucose utilization, and lower glucose utilization might contribute to apoptosis induction. Furthermore, glucose utilization regulated the switch between senescence and apoptosis, and p53 played an important role in this process (Figure 5F).

Discussion

In this study, our results showed that PAB-induced senescent cells exhibit higher glucose utilization in p53 wild-type A549 and H460 cells. However, the utilization of glucose was decreased in PAB-induced apoptotic H1299 cells (p53-null). These data suggest that cells undergoing mitotic catastrophe enter into different fates (senescence or apoptosis) because of the status of p53 to regulate higher or lower glucose utilization. Further studies also demonstrate that blocking the utilization of glucose could elevate the apoptosis ratio in PAB-treated p53 wild-type cells.

Cellular senescence is a state of irreversible cell cycle arrest with active metabolism[19]. Drug-induced senescence might be a desirable therapeutic strategy, especially for tumors with

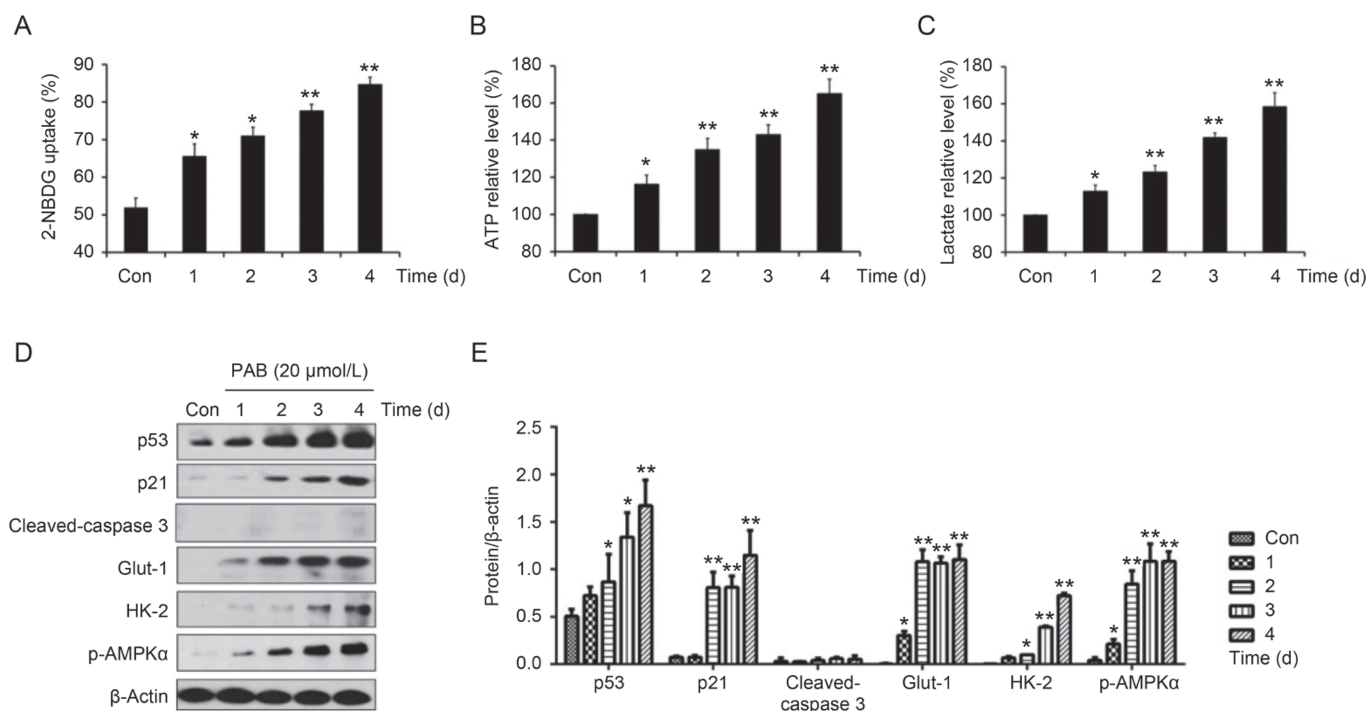

Figure 4A–4E. Glucose utilization regulated the switch between senescence and apoptosis in PAB-treated H460 cells. (A–E) Cells were incubated with 20 μmol/L PAB for the indicated time periods between d 0 and d 4. (A) 2-NBDG uptake by flow cytometry was measured. (B, C) The relative ATP and lactate levels were examined by commercial ATP (B) and lactate (C) assay kits, respectively. The data are presented as the mean±SD of three independent experiments. $^{*}P<0.05$, $^{**}P<0.01$ *vs* the control group. (D) The levels of p53, p21, caspase 3, Glut-1, HK-2 and p-AMPKα (Thr172) were evaluated by Western blotting. (E) The band densities of proteins were calculated using ImageJ software.

apoptosis resistance, but there are some potentially harmful properties in viable senescent cells[20]. After chemotherapy, senescence-competent lymphomas show increased glucose utilization and much higher ATP production[20]. Similar effects are observed in PAB-caused senescent cells.

Autophagy, a lysosome-dependent degradation pathway, promoted glucose uptake and glycolytic flux[21]. In some cases, autophagy inhibition could switch camptothecin-induced senescence to apoptosis in cancer cells[22]. Moreover, emerging evidence also demonstrates that apoptosis induction in tumor cells may be closely associated with the inhibition of glycolysis[23]. Our previous study indicated that the knockdown of p53 or p21 increased apoptotic cell death in PAB-treated A549 cells. Therefore, we speculated that the knockdown of p53 (or p21) might impair the autophagy process and lead to lower glucose utilization with the decrease in Glut-1, HK-2 and p-AMPKα levels. Meanwhile, the decreased energy demand was accompanied with AMPK inactivation[24]. The detailed mechanism should be further studied.

Under some conditions, glucose withdrawal combined with antitumor drugs induces much more cancer cell death[25]. By contrast, cells cultured in high-glucose medium sometimes promote the senescence process[26,27]. Although metabolism is involved directly or indirectly in any cell, the examination of how metabolism is regulated in proliferating cells would provide new insight. The metabolism of glutamine, another major nutrient consumed by tumor cells besides glucose, has also been considered as a target for cancer therapy. Deficiency in glutamine, but not in glucose, induces proto-oncogene Myc-dependent apoptosis in human cells[28].

AMPK, which is found in all eukaryotes, is an evolutionarily conserved sensor of the cellular energy status. AMPK activity is regulated by the cellular AMP/ATP ratio and upstream kinases[29]. It couples cellular bioenergetics to metabolic control and cell growth[30]. The activation of AMPK indicates that cells exhibit enhanced energetic demands[24]. Several bodies of evidence have suggested that the regulation of AMPK has been considered as a target for cancer therapy[31]. Senescent cells were susceptible to the inhibition of the energy sensor AMPK[20].

Cells deficient in ATP often undergo apoptosis[32]. The inhibition of glycolysis may severely abolish ATP generation in cancer cells and thus may preferentially kill the tumor cells[33]. However, a potential problem is that the glycolytic inhibitors currently available are not very potent; hence, high doses are required[7]. 2-DG is a relatively specific blocker of glycolysis without altering other nutrients or metabolic pathways[34]. 2-DG alone cannot induce obvious cytotoxicity between 0.5 and 5 mmol/L in non-small cell lung cancer cells, while it enhances other antitumor drug-induced cell death[35,36]. Our

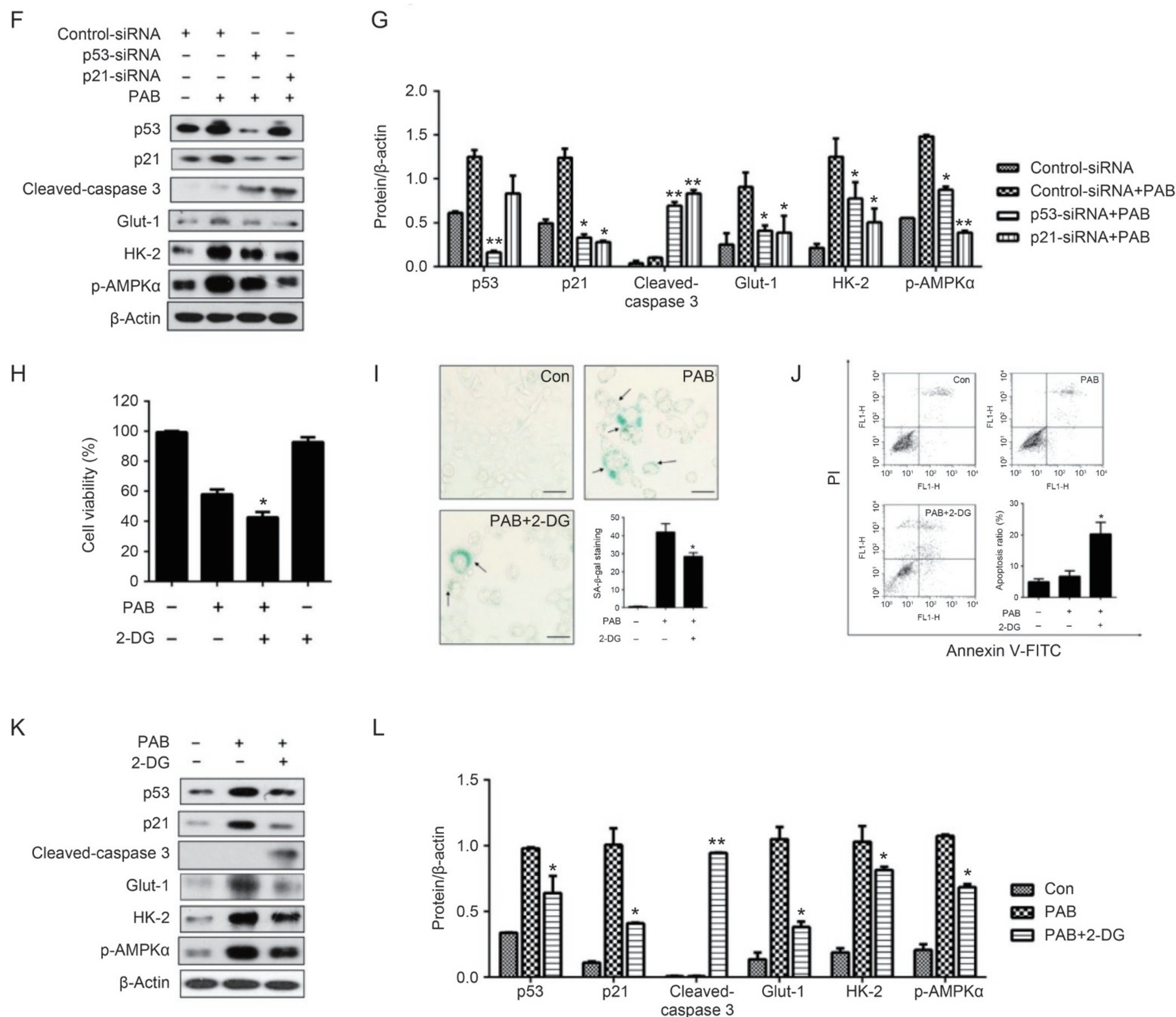

Figure 4F–4L. H460 cells were transfected with control, p53 or p21 siRNA for 24 h. (H-L) Cells were incubated with PAB (20 μmol/L) or combined with 2-DG (1 mmol/L) for 3 d. (H) Cell viability was examined by the MTT assay. (I) Senescent cells were observed by SA-β-gal staining. (J) Annexin V/PI staining was applied to detect the apoptosis ratio. (K, L) The band densities of proteins were calculated using ImageJ software. The data are presented as the mean±SD of three independent experiments. **P*<0.05, ***P*<0.01 *vs* the PAB-treated group.

investigation revealed that the combination of PAB with a glycolytic inhibitor (2-DG) converts senescence into apoptosis. Thus, PAB can induce apoptosis in A549 and H460 cells with glycolysis inhibition. Therefore, the elimination of senescent tumor cells can enhance the efficiency of cancer therapy. However, there is still much to learn about how tumor cell metabolism is regulated.

P53, a well-known tumor suppressor, plays critical roles in the regulation of cellular processes, including apoptosis and senescence[37,38]. However, PAB induced p53-dependent senescence but p53-independent apoptosis in human lung cancer cells[15]. Recent studies have also revealed that p53 possesses glucose metabolic function[39]. In some cases, p53 downregulates the activity of glucose-6-phosphate dehydrogenase (G6PD)[40] or represses glucose transport genes[41] in tumor cells. In this study, PAB caused enhanced glucose utilization, which resists the induction of apoptosis. This effect might be associated with the drawbacks of p53-dependent senescence. A growing body of evidence has suggested that p53 provides mechanisms to overcome metabolic stress and might contribute to tumor survival[42,43].

The role of p53 in senescence induction has been well documented in tumor cells as a response to chemotherapy. In some studies, chemotherapeutic drugs were found to induce

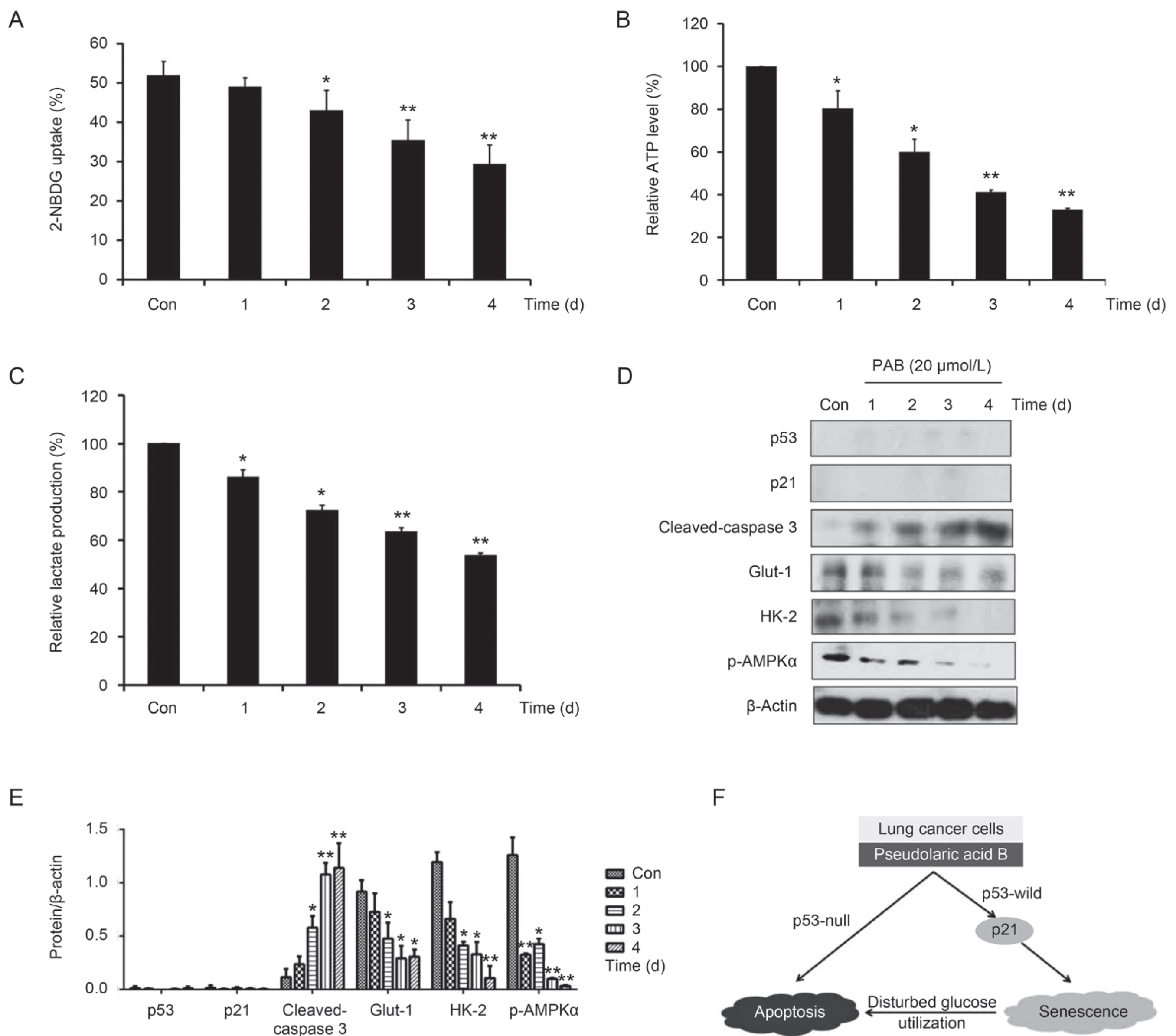

Figure 5. PAB decreased the utilization of glucose in H1299 cells. (A–D) H1299 cells were incubated with 20 μmol/L PAB for 0–4 d. (A) The glucose uptake ratio was indicated by flow cytometric analysis with 2-NBDG staining. (B, C) Commercial ATP and lactate assay kits were used to measure the levels of ATP (B) and lactate (C). (D) The expression levels of p53, p21, caspase 3, Glut-1, HK-2 and p-AMPKα (Thr172) were examined by Western blotting. (E) The band densities of proteins were quantified by ImageJ software. (F) A schematic diagram of glucose utilization in the regulation of PAB-induced senescence and apoptosis in p53 wild-type and -null human lung cancer cells. The data are presented as the mean±SD of three independent experiments. $^{*}P<0.05$, $^{**}P<0.01$ *vs* the control group.

senescence in p53 wild-type cell lines, whereas p53 mutation or inactivation would result in the transition from senescence to apoptosis[44-49]. Additionally, several reports have shown that both wild-type and mutant p53 are involved in glucose metabolism, but with opposite effects[50]. Moreover, the AMPK activity was also inhibited in p53 mutant cell lines[51,52]. Thus, we speculated that PAB can exhibit similar effects in the H1299 cell line in p53 mutant lung cancer cells. Oridonin, an activated diterpenoid isolated from *Rabdosia rubescens*, caused cell death as well as glucose metabolism inhibition in p53-mutated colorectal cancer cells[53].

The present study has identified a new underlying mechanism by which PAB induces a switch from senescence to apoptosis by blocking the utilization of glucose. The combination of glycolytic inhibition and other anticancer agents could provide a preferential method to induce tumor cell death with fewer side effects.

Author contribution

Guo-dong YAO and Takashi IKEJIMA designed the research;

Guo-dong YAO, Jing YANG, Xiu-xiu LI and Xiao-yu SONG performed the research; Shin-Ichi TASHIRO and Satoshi ONODERA contributed some reagents; Guo-dong YAO analyzed the data and wrote the paper; Toshihiko HAYASHI, Shao-jiang SONG and Takashi IKEJIMA revised the paper.

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