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Metformin Suppresses Proliferation and Viability of Rat Pheochromocytoma Cells

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

AB **Min Li***
E **Xiuli Jiang***
E **Tingwei Su**
C **Lei Jiang**
CD **Weiwei Zhou**
AG **Weiqing Wang**

Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Key Laboratory for Endocrine Tumors, Rui-Jin Hospital, Shanghai Jiao-Tong University School of Medicine, Shanghai, P.R. China

* Min Li and Xiuli Jiang equally contributed to this work

Corresponding Author: Weiqing Wang, e-mail: wqingw61@163.com

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Background: Previous studies have clearly demonstrated that metformin inhibits cell proliferation and cell growth in many types of human cancers. Increased survival rates in patients with breast and lung cancer receiving metformin have also been observed. However, the effect of metformin on pheochromocytoma cells remains unexplored.

Material/Methods: Rat pheochromocytoma cells (PC12 cells) were cultured and treated with metformin or vehicle control. Cell proliferation, cell-cycle, apoptosis, genes expression, and the signaling pathways involved were analyzed in PC12 cells.

Results: The metformin treatment reduced cell viability and proliferation in rat pheochromocytoma PC12 cells in a dose- and time-dependent manner. Furthermore, metformin exposure led to an increased apoptosis rate and cell-cycle arrest accompanied with downregulation of *Ccna2* and *Ccnb2*. At the molecular level, the AMPK signaling pathway was activated, whereas the mTOR and ERK1/2 signaling pathways were inhibited by metformin.

Conclusions: Our data suggest an antiproliferative role of metformin in pheochromocytoma development, which may provide a novel option for future cancer therapy.

MeSH Keywords: **AMP-Activated Protein Kinases • Cell Proliferation • Metformin • Pheochromocytoma • TOR Serine-Threonine Kinases**

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Background

Pheochromocytoma, a neuroendocrine tumor originating in the medulla of the adrenal glands, usually causes paroxysmal hypertension, palpitations, diaphoresis, and weight loss, due to elevated catecholamine levels [1]. Genetic studies have demonstrated that mutations of several genes could increase the risk of pheochromocytoma, such as multiple endocrine neoplasia 2A, 2B (*MEN2A* and *MEN2B*), and von Hippel-Lindau (*VHL*) [2–4]. Although surgical resection of the tumor is the first choice of treatment, such surgery should be performed only at centers experienced in the management of this serious disorder. Therefore, an alternative candidate for its prevention and treatment is urgently required.

Metformin is one of the most widely used antihyperglycemic drugs and insulin sensitizers, which inhibits hepatic glucose production and enhances peripheral glucose uptake [5,6]. Interestingly, recent studies found that metformin has antiproliferative activity in many types of human malignancies, including breast, gastric, colon, and prostate, and renal cancers [7–10]. In addition, several retrospective reports showed that metformin is also associated with a decreased risk of developing cancer and cancer-related mortality [11]. Indeed, *in vitro* and *in vivo* analyses showed that metformin modulates many molecular pathways, including the activation of AMPK signaling and suppression of mTOR and PI3K/ATK signaling [12–14]. Furthermore, the expression of cell-cycle regulators, such as *Ccnd1*, *Cdkn1b*, and *Cdkn1c*, was also inhibited by metformin treatment [15–17]. However, whether metformin inhibits the development and progression of pheochromocytoma remains unknown.

In the present study, we aimed to examine whether metformin exerts an antitumor effect in PC12 cells, a rat pheochromocytoma cell line, and to analyze the underlying mechanisms.

Material and Methods

Ethics statement

This study was reviewed and approved by the Research Ethics Committee of Rui-jin Hospital, Shanghai Jiao-Tong University School of Medicine (Shanghai, China).

Cell culture and treatment

Rat pheochromocytoma cells (PC12 cells) were purchased from the Shanghai Institute of Biological Science (SIBS, Shanghai, China). The cells were maintained in RPMI-1640 (GBICO, Rockville, MD, USA) with 10% horse serum and 5% fetal calf serum at 37°C with infusion of 5% CO_2 and humidified air. Metformin (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in milliQ water and applied at concentrations from 1 to 50 mmol/L. PC12 cells were cultured for 24 h, followed by treatment with metformin for different times and doses.

Cell viability and proliferation assays

PC12 cells at a density of 3×10^3 per well were seeded on 96-well plates. PC12 cell proliferation curves were observed following the protocol of CCK-8 (Cell Counting Kit-8, Dojindo Molecular Technologies, Japan) after the application of different doses and times of treatment with metformin. Further, CCK-8 analysis was performed on day 3, when the cells were in the stationary phase of growth, to determine the survival rate of PC12 cells in the treatments with metformin or vehicle. The final absorbance of each well was determined at a wavelength of 450 nm using a microplate reader (Bio-Rad Laboratories, Shanghai, China).

Flow cytometry experiments

To analyze cell proliferation, EdU staining was performed using Click-iT® EdU Flow Cytometry Assay Kits (Invitrogen, Shanghai, China) according to the manufacturer's protocols. Dispersed cells were plated in a 6-well dish. Forty-eight hours after the treatment with metformin, the cells were incubated with 10 uM EdU for another 4 h. Then, the cells were fixed and permeabilized, followed by EdU detection and treatment with PI staining before the analysis. To analyze cell-cycle progression, cells were harvested and immobilized in 70% ethanol at 4°C for 12 h, followed by washing with phosphate buffer solution (PBS). Then, the cells were incubated with RNase (10 µg/mL), resuspended in propidium iodide (50 µg/mL), and placed in a dark room. To analyze cell apoptosis, cells were stained with the Annexin V-FITC reaction reagent (5 µL of Annexin V-FITC and 5 µL of propidium iodide) and detected using an Accuri C6 flow cytometer.

RNA extraction and quantitative real-time PCR

A TRIzol Kit (Invitrogen, Shanghai, China) was used to extract total RNA from PC12 cells following the manufacturer's protocols. cDNA was converted from 1 mg of total RNA by Reverse Transcription System (Promega, Madison, WI, USA). Quantitative real-time PCR analysis was conducted using SYBR Premix Ex Taq reagents (Takara, Shiga, Japan), and calculations were performed using the $2^{-\Delta\Delta}$Ct method. Rn18s were employed to normalize target genes expression. The gene primers were listed as follows:
Ccna2: F: 5'-GATGCCCTGGCTTTTAGTGC-3';
R: 5'-CATTCACTGGCTT TTCGTCTTC-3';
Ccnb2: F: 5'-GACCGGCTCAAGTGGCTAAG-3';
R: 5'-TCAGAG AAAGCTTGGCAGAGG-3';

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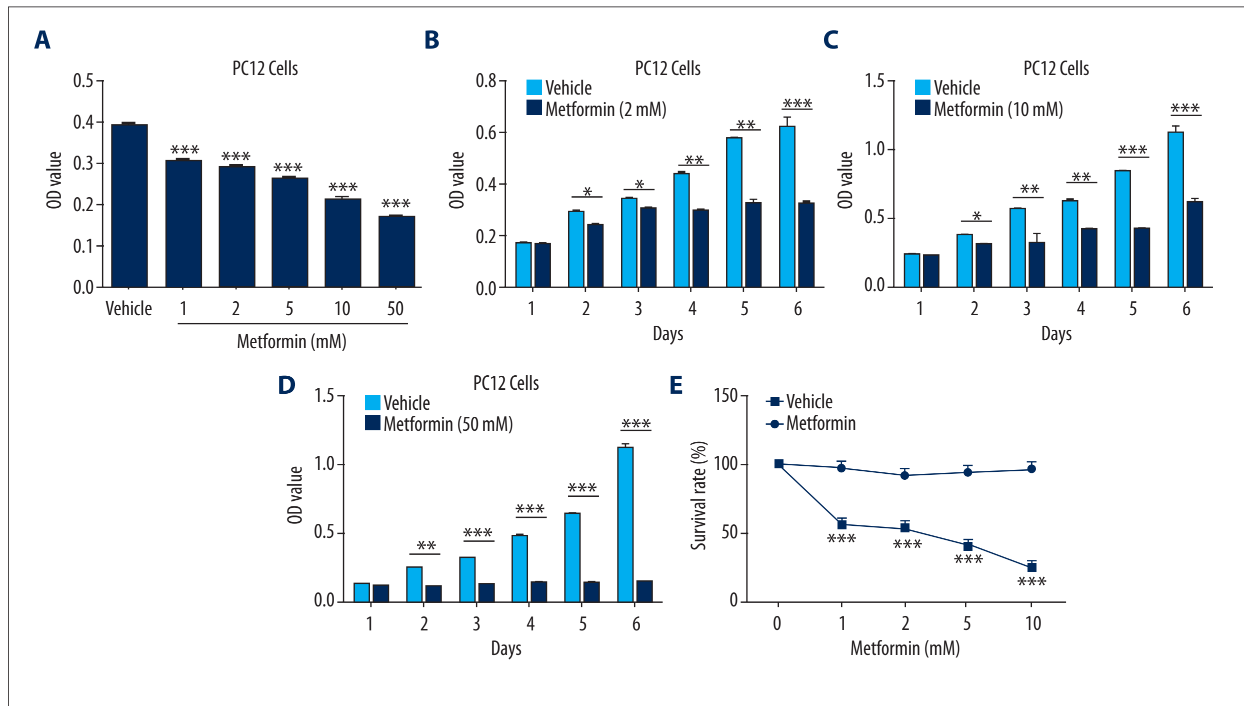

Figure 1. Cell growth of PC12 cells. (**A**) Cell growth of PC12 cells treated with different doses of metformin (1, 2, 5, 10, and 50 mM) or vehicle for 48 h was analyzed by CCK-8 assay. (**B–D**) Cell growth of PC12 cells treated with different doses of metformin (2, 10, and 50 mM) or vehicle for different time periods (Day 1 to Day 6) analyzed by CCK-8 assay. (**E**) Cell survival rate of PC12 cells treated with different doses of metformin or vehicle analyzed by CCK-8 assay. The proliferation and survival rates of PC12 cells were decreased by metformin in a dose- and time-dependent manner.

PCNA: F: GGCGTGAACCTACAGAGC AT-3';
R: 5'- CACAGGAGATCACCACAGCA-3';
Cdkn1b: F: 5'-GTGTCCAGGGATGAGGAG C-3';
R: 5'-TCGGAGCTGTTTACGTCTGG-3';
Cdkn1c: F: 5'-TCGGAGCTGTTTAC GTCTGG-3';
R: 5'-CTGAGCAGGTCTCTGAGCA-3';
Ccnd1: F: 5'-CGCACAACGC ACTTTCTTTC-3';
R: 5'-TTGGGATCGATGTTCT-GCTG-3';
Ccnd3: F: 5'-AACCAC GCCCCTGACTATTG-3';
R: 5'-GGTCACTGGGCAGAGAGAGG-3';
Rn18s: F: 5'-A TTCGAACGTCTGCC CTATCAA-3';
R: 5'-CGGGAGTGGGTAATTTGC G-3'.

Western blotting

Total proteins were prepared with an ice-cold lysis buffer containing 50 mM of Tris-HCl, 100 mM of 2-Mercaptoethanol, 2% w/v SDS, and 10% glycerol. Then, separation by 6% or 10% sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) was done, transferred onto polyvinylidene fluoride (PVDF) membranes, followed by blocking by 10% bovine serum albumin (BSA). Next, the proteins were incubated with the following primary and secondary antibodies: ERK1/2, mTOR, AMPK, and GAPHD (Cell Signaling Technology, Beverly, MA, USA).

Statistical analysis

Results from at least 3 independent experiments were analyzed by GraphPad Prism software (GraphPad Software, version 6.01) and expressed as mean ± standard error of the mean (SEM). The significant differences were determined using the Student's *t*-test for two-group comparisons or the one-way ANOVA for multi-group comparisons. Statistical significance was accepted at * $P<0.05$, ** $P<0.01$, or *** $P<0.001$.

Results

Metformin suppressed the viability and proliferation of PC12 cells

To explore whether metformin affects the growth of pheochromocytoma cells, PC12 cells were exposed to different doses of metformin or vehicle. Cell growth was inhibited after 72 h of metformin treatment in a dose-dependent manner (Figure 1A). Given the strong inhibitory effects of metformin at concentrations of 2, 10, and 50 mM, time-course experiments were performed using these doses. As shown in Figure 1B–1D, the cell growth gap was enlarged from day 1 to day 6, which verified the time-dependent function of metformin. Furthermore,

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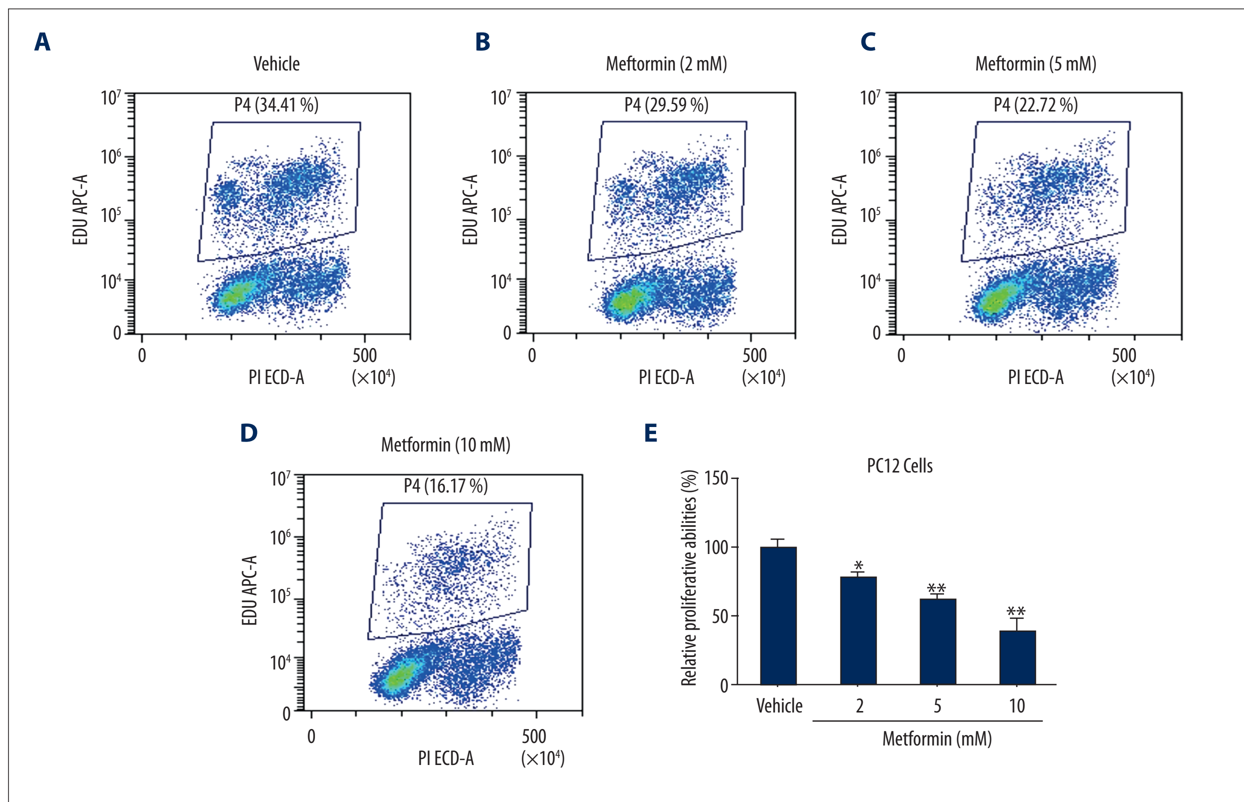

Figure 2. Cell proliferation ability of PC12 cells. (**A–D**) Cell proliferative ability of PC12 cells treated with 3 doses of metformin (2, 5, and 10 mM) or vehicle were analyzed by EDU and flow cytometry. The apoptosis induced by metformin was dose-dependent. (**E**) Statistical histogram of the relative cell proliferative ability of PC12 cells.

cell viability was significantly inhibited by the different doses of metformin, as evidenced by CCK-8 assays (Figure 1E). Consistently, the EdU flow cytometry analysis showed that the cell proliferation potential was suppressed by metformin treatment (Figure 2A–2E). These data suggest an inhibitory effect of metformin on the growth of pheochromocytoma cells.

Metformin caused cell-cycle arrest and increased cell apoptosis of PC12 cells

Next, cell-cycle progression and cell apoptosis were analyzed in PC12 cells administered with metformin or vehicle. Flow cytometry analysis also revealed a cell-cycle arrest at the G0/G1 phase of the PC12 cells treated with metformin (Figure 3A–3E). For apoptosis analysis, flow cytometry was performed after staining with Annexin V/PI, showing that the metformin treatment induced a higher apoptosis rate (Figure 4A–4E). Most importantly, the cell-cycle and apoptosis of PC12 cells were inhibited by treatment with metformin.

Modulation of cell-cycle regulators by metformin

Given that metformin inhibits cell-cycle progression in PC12 cells, we speculated that expression of cell-cycle regulators might be modulated by metformin treatment. Therefore, the mRNA levels of genes responsible for cell-cycle progression were determined by quantitative real-time PCR. Among them, *Ccna2, Ccnb2*, and *PCNA* were significantly downregulated by metformin treatment (Figure 5A), whereas other cell-cycle regulators, including *Cdkn1b*, *Cdkn1c*, *Ccnd1*, and *Ccnd3,* remained unaffected (Figure 5B). Therefore, the expression levels of certain cell-cycle regulators may be modulated by metformin treatment of PC12 cells.

Signaling pathways affected by metformin

It has been shown that several pathways are involved in the execution of the suppressive function of metformin on tumor growth, such as the activation of AMPK signaling and suppression of mTOR signaling [12–14]. The Western blot analyses in our study revealed that AMPK phosphorylation was enhanced in PC12 cells treated with metformin (Figure 6A, 6B). Moreover, phosphorylated ACC, a downstream target of AMPK, was also induced by metformin (Figure 6A, 6B). On the other hand, phosphorylated mTOR and ERK1/2 levels were reduced (Figure 6A, 6B), suggesting that these downstream signaling pathways were inhibited in the PC12 cells upon metformin treatment. Therefore, the regulation of AMPK, mTOR, and

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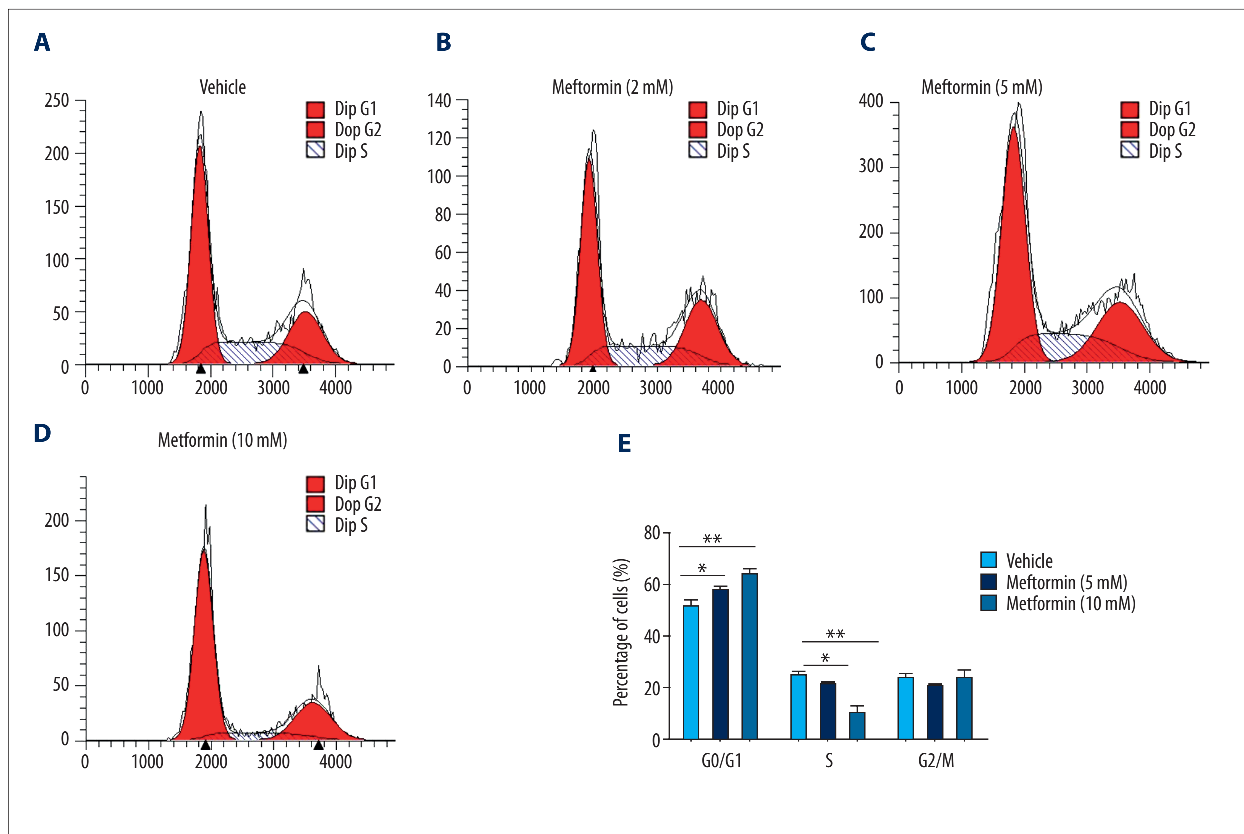

Figure 3. Cell-cycle analysis of PC12 cells. (**A–D**) Cell-cycle analysis by flow cytometry of PC12 cells treated with vehicle (**A**) or metformin (**B**: 2 mM; **C**: 5 mM; and **D**: 10 mM). (**E**) Representative histogram data of the cell-cycle analysis of PC12 cells treated with vehicle or metformin (5 and 10 mM). PC12 cells were inhibited at G0/G1 phase by metformin.

ERK1/2 pathways might be responsible for the antitumor effects of metformin on pheochromocytoma cells.

Discussion

Although the biological roles of metformin have been explored in many types of human cancers, its effect on the growth of pheochromocytoma cells remains poorly understood. In the present study, using *in vitro* models, we showed that metformin inhibited cellular proliferation in rat pheochromocytoma cells. This finding is supported by several lines of evidence. First, cell growth rate and proliferation ability were decreased in a dose- and time-dependent manner after treatment with metformin. Second, the cell-cycle was arrested in the G0/G1 phase, accompanied by changes in the expression of related genes. Third, Annexin V/PI staining analysis confirmed that metformin treatment increased the cell apoptosis rate. Therefore, the remarkable suppressive efficiency of metformin on pheochromocytoma cell proliferation and growth might have important implications for the treatment of this serious disease. However, *in vivo* studies, such as xenograft models, are needed to further determine the antiproliferative effect of metformin on pheochromocytoma cells.

At the molecular level, our results indicate that *Ccna2* and *Ccnb2*, which are 2 critical regulators of cell-cycle progression, are downregulated by metformin and may be intracellular targets of its antiproliferative activities. *Ccna2* is expressed at the onset of S phase and is involved in the G2/M transition [18], whereas *Ccnb2* was shown to control the proper timing of centrosome separation and transforming growth factor beta-mediated cell-cycle transition [19,20]. It has been well-established that the expression levels of these 2 cyclin proteins are significantly upregulated in many types of human cancers, suggesting that their inhibition might be beneficial to the control of cancer cell proliferation. It should be noted that our data suggest that other important cell-cycle regulators, including *Ccnd1*, *Cdkn1b,* and *Cdkn2c*, were not affected by metformin treatment. Previous studies have shown that metformin exerts an antitumor effect in breast and prostate cancer cells through a decrease of cyclin D1 level [21]. Cai et al. reported that metformin induced G0/G1 phase arrest in esophageal squamous cell carcinomas, which was accompanied by the upregulation of p21 and p27 [22]. Moreover, Rodríguez-Lirio et al. demonstrated that metformin blocked leukemia cell proliferation by inducing arrest of S and G2/M phases through downregulation of cyclin A and cyclin B1, but not that of cyclin E [23].

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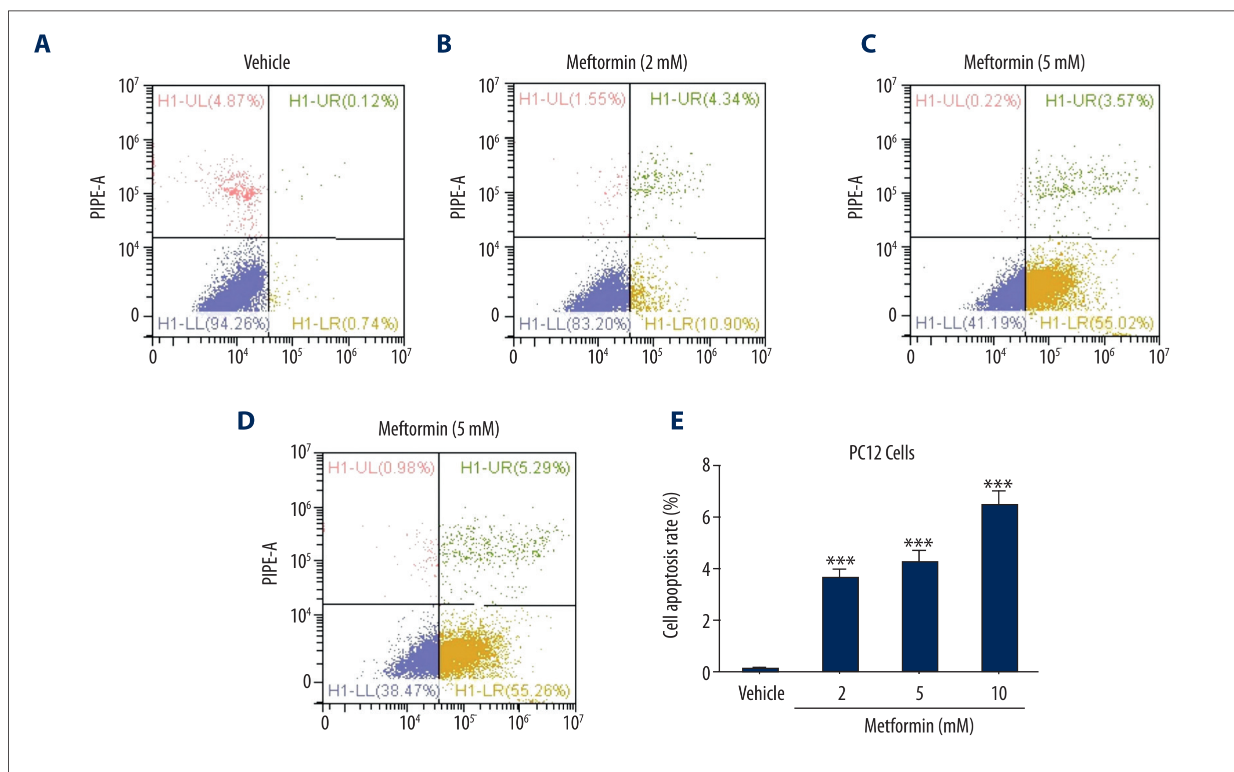

Figure 4. Cell apoptosis in PC12 cells. (**A–D**) Cell apoptosis analysis performed by Annexin V/PI staining and flow cytometry in PC12 cells treated with 3 doses of metformin (2, 5, and 10 mM) or vehicle. (**E**) Statistical histogram of cell apoptosis rate of PC12 cells. Apoptosis rates of PC12 cells were increased by metformin.

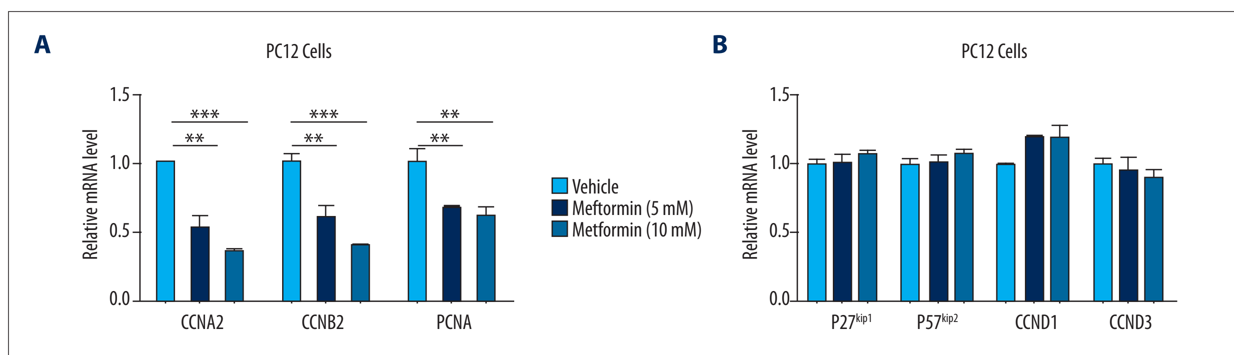

Figure 5. Expression levels of cell-cycle regulators in PC12 cells. (**A**) Relative mRNA levels of *Ccna2, Ccnb2*, and *PCNA* in PC12 cells treated with 3 doses of metformin (2, 5, and 10 mM) or vehicle for 48 h. (**B**) Relative mRNA levels of *Cdkn1b*, *Cdkn1c*, *Ccnd1*, and *Ccnd3* in PC12 cells treated with 3 doses of metformin (2, 5, and 10 mM) or vehicle for 48 h. The expression levels of cell-cycle related genes were regulated by metformin.

Although the reason for this inconsistency remains unknown, we speculate that the molecular targets of metformin might be cell- or tissue-specific.

Mechanistically, initial studies found that metformin activates the AMPK pathway, which is essential for the antiproliferative effects of metformin [24]. However, subsequent studies showed that genetic inhibition of the AMPK pathway did not inhibit the effect of metformin, suggesting that additional mechanisms are involved [25]. For instance, it has been shown that metformin regulates several intracellular signaling pathways, including mTOR, ERK, and PI3K/AKT. Here, we found that metformin also activates phosphorylated AMPK, whereas it inhibits phosphorylated mTOR and ERK1/2. Therefore, we speculate that the effect of metformin on pheochromocytoma cell growth, at least in part, is dependent on the modulation of these signaling pathways.

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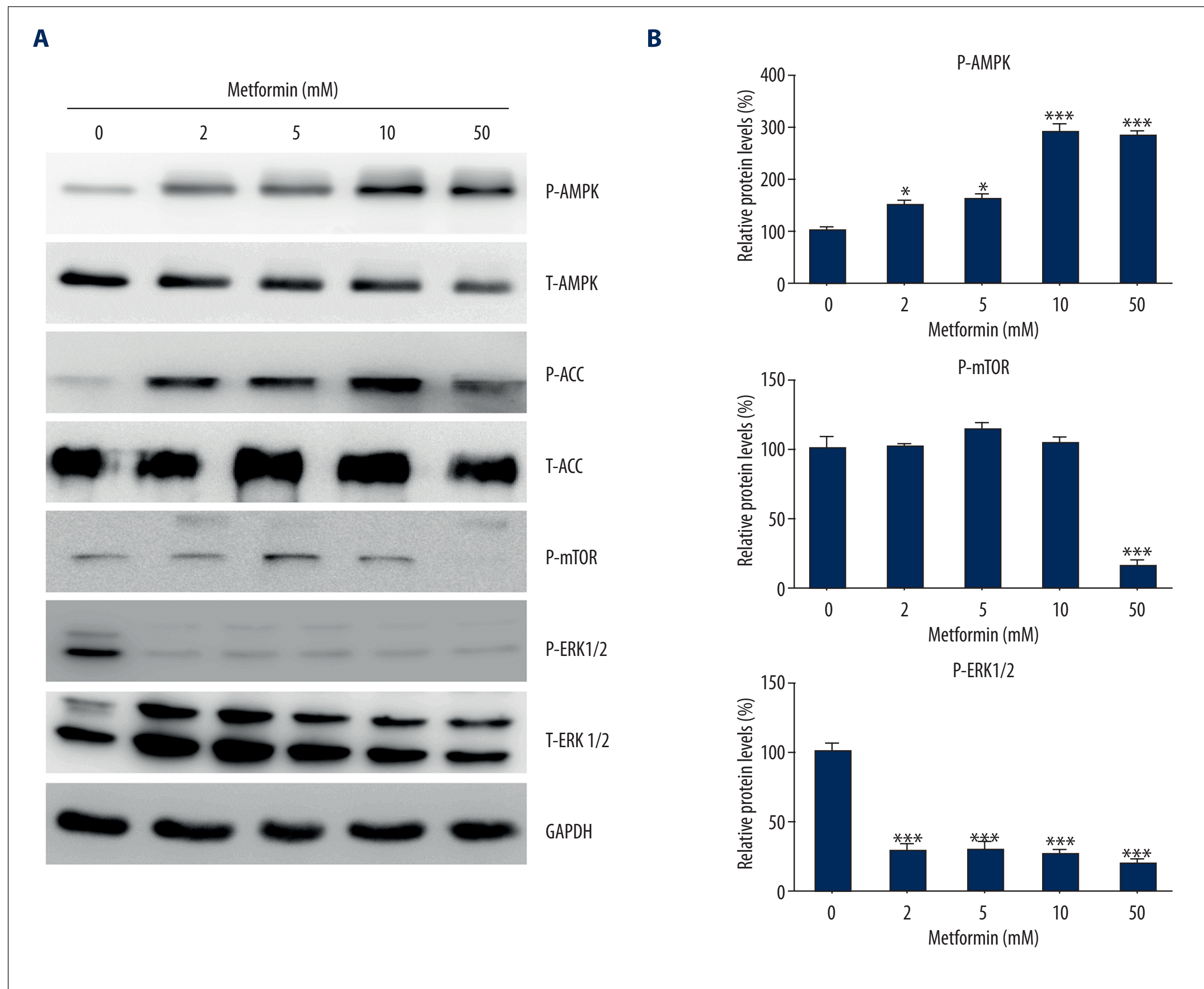

Figure 6. Downstream signaling pathways in PC12 cells. (**A**) Representative protein levels of phosphorylated AMPK, mTOR, and ERK1/2 in PC12 cells treated with metformin or vehicle control, as indicated. Total AMPK, mTOR, ERK1/2, and GAPDH were used as loading controls. (**B**) Statistical histogram of the relative protein levels of phosphorylated AMPK, mTOR, and ERK1/2. AMPK phosphorylation was activated by metformin in PC12 cells, whereas the activation of mTOR and ERK1/2 was inhibited.

In summary, we evaluated the antiproliferative effects of metformin in pheochromocytoma cells. Given that metformin has a superior safety profile and few adverse effects [26], it will be of crucial importance to determine its effects in patients with pheochromocytoma.

Conclusions

Metformin treatment reduced cell viability and proliferation ability of PC12 cells in a dose- and time-dependent manner. Metformin also increased the apoptosis rate and induced cell-cycle arrest, accompanied by downregulation of *Ccna2* and *Ccnb2*. At the molecular level, AMPK, mTOR, and ERK1/2 signaling pathways were regulated by metformin. Taken together, our data suggest a previously unknown role of metformin in pheochromocytoma development, which might provide a novel option for future cancer therapy.

Conflict of interest

None.

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References:

1. Favier J, Amar L, Gimenez-Roqueplo AP: Paraganglioma and phaeochromocytoma: From genetics to personalized medicine. Nat Rev Endocrinol, 2015; 11(2): 101–11
2. Bravo EL, Tagle R: Pheochromocytoma: State-of-the-art and future prospects. Endocr Rev, 2003; 24(4): 539–53
3. Dahia PL: Pheochromocytoma and paraganglioma pathogenesis: Learning from genetic heterogeneity. Nat Rev Cancer, 2014; 14(2): 108–19
4. Burnichon N, Buffet A, Gimenez-Roqueplo AP: Pheochromocytoma and paraganglioma: Molecular testing and personalized medicine. Curr Opin Oncol, 2016; 28(1): 5–10
5. Madiraju AK, Erion DM, Rahimi Y et al: Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. Nature, 2014; 510(7506): 542–46
6. Miller RA, Chu Q, Xie J et al: Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. Nature, 2013; 494(7436): 256–60
7. Daugan M, Dufaÿ Wojcicki A, d'Hayer B et al: Metformin: An anti-diabetic drug to fight cancer. Pharmacol Res, 2016; 113(Pt A): 675–85
8. Ma SJ, Zheng YX, Zhou PC et al: Metformin use improves survival of diabetic liver cancer patients: Systematic review and meta-analysis. Oncotarget, 2016; 7(40): 66202–11
9. Sośnicki S, Kapral M, Węglarz L: Molecular targets of metformin antitumor action. Pharmacol Rep, 2016; 68(5): 918–25
10. Xie W, Wang L, Sheng H et al: Metformin induces growth inhibition and cell cycle arrest by upregulating MicroRNA34a in renal cancer cells. Med Sci Monit, 2017; 23: 29–37
11. Chae YK, Arya A, Malecek MK et al: Repurposing metformin for cancer treatment: current clinical studies. Oncotarget, 2016; 7(26): 40767–80
12. Li S, Han D, Zhang Y et al: Activation of AMPK prevents monocrotaline-induced extracellular matrix remodeling of pulmonary artery. Med Sci Monit Basic Res, 2016; 22: 27–33
13. Dowling RJ, Zakikhani M, Fantus IG et al: Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells. Cancer Res, 2007; 67(22): 10804–12
14. Peng M, Huang Y, Tao T et al: Metformin and gefitinib cooperate to inhibit bladder cancer growth via both AMPK and EGFR pathways joining at Akt and Erk. Sci Rep, 2016; 6: 28611
15. Gwak H, Kim Y, An H et al: Metformin induces degradation of cyclin D1 via AMPK/GSK3β axis in ovarian cancer. Mol Carcinog, 2017; 56(2): 349–58
16. Li P, Zhao M, Parris AB et al: p53 is required for metformin-induced growth inhibition, senescence and apoptosis in breast cancer cells. Biochem Biophys Res Commun, 2015; 464(4): 1267–74
17. Xu Y, Lu S: Metformin inhibits esophagus cancer proliferation through upregulation of USP7. Cell Physiol Biochem, 2013; 32(5): 1178–86
18. Yasmeen A, Berdel WE, Serve H, et al: E- and A-type cyclins as markers for cancer diagnosis and prognosis. Expert Rev Mol Diagn, 2003; 3(5): 617–33
19. Nam HJ, van Deursen JM: Cyclin B2 and p53 control proper timing of centrosome separation. Nat Cell Biol, 2014; 16(6): 538–49
20. Liu JH, Wei S, Burnette PK et al: Functional association of TGF-beta receptor II with cyclin B. Oncogene, 1999; 18(1): 269–75
21. Ben Sahra I, Laurent K, Loubat A et al: The antidiabetic drug metformin exerts an antitumoral effect *in vitro* and *in vivo* through a decrease of cyclin D1 level. Oncogene, 2008; 27(25): 3576–86
22. Peyton KJ, Liu XM, Yu Y, et al: Activation of AMP-activated protein kinase inhibits the proliferation of human endothelial cells. J Pharmacol Exp Ther, 2012; 342(3): 827–34
23. Rodríguez-Lirio A, Pérez-Yarza G, Fernández-Suárez MR et al: Metformin induces cell cycle arrest and apoptosis in drug-resistant leukemia cells. Leuk Res Treatment, 2015; 2015: 516460
24. Zakikhani M, Dowling R, Fantus IG et al: Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells. Cancer Res, 2006; 66(21): 10269–73
25. Ben Sahra I, Regazzetti C, Robert G et al: Metformin, independent of AMPK, induces mTOR inhibition and cell-cycle arrest through REDD1. Cancer Res, 2011; 71(13): 4366–72
26. Steber CJ, Perkins SL, Harris KB: Metformin-Induced fixed-drug eruption confirmed by multiple exposures. Am J Case Rep, 2016; 17: 231–34

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