Metformin Inhibit Lung Cancer Cell Growth and Invasion in Vitro as Well as Tumor Formation in Vivo Partially by Activating PP2A

Xiaohu Zhou*
Shanshan Liu*
Xuemei Lin
Liping Xu
Xiaoming Mao
Jun Liu
Zixing Zhang
Wenhong Jiang
Hua Zhou

* Xiaohu Zhou and Shanshan Liu contributed to this work equally

Background: The aim of this study was to investigate whether PP2A activation is involved in the anti-cancer activity of metformin.

Material/Methods: A549 and H1651 human lung cancer cells were constructed with stable α4 overexpression (O/E α4) or knock-down of PP2A catalytic subunit A/B (sh-PP2Ac). Influences of okadaic acid (OA) treatment, O/E α4 or sh-PP2Ac on metformin treated cells were investigated by cell viability, proliferation, apoptosis, and Transwell invasion assay in vitro. Protein expression levels of Bax, Bcl-2, Myc, and Akt as well as serine phosphorylation level of Bax, Myc, and Akt were examined by western blot. For in vivo assays, wild type (WT) or modified A549 cells were subcutaneously injected in nude mice, and metformin treatment on these xenografted tumors were assayed by tumor formation assay and western blot detecting cell proliferation marker PCNA (proliferating cell nuclear antigen) as well as protein expression level and serine phosphorylation level of Akt and Myc.

Results: Metformin treatment significantly reduced A549 or H1651 cell growth and invasive capacity in vitro as well as Ser184 phosphorylation of Bax, Ser62 phosphorylation of Myc, and Ser473 phosphorylation of Akt, all of which could be partially attenuated by OA treatment, O/E α4 or sh-PP2Ac. Metformin treatment also significantly reduced tumor formation in vivo as well as protein expression of PCNA, Akt, Myc, and serine phosphorylation of the latter 2, which can be partially blocked by O/E α4 or sh-PP2Ac.

Conclusions: Metformin reduced lung cancer cell growth and invasion in vitro as well as tumor formation in vivo partially by activating PP2A.

MeSH Keywords: Lung Neoplasms • Metformin • Mycolic Acids • Phosphorylation

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/912059
Background

Lung cancer is currently the second most often diagnosed cancer type and leading cause of cancer-related death over the globe [1]. Aside from surgery, therapy against lung cancer has evolved from chemotherapy to targeted therapy and immunotherapy, greatly improved prognosis and survival of patients diagnosed with early-to-mid stage lung cancer, but survival of those with late stage lung cancer remains poor [1,2]. Recent findings have revealed the novel anti-cancer activity of a conventional hypoglycemic drug for anti-diabetic purpose, metformin, against lung cancer and many other cancer types [3,4]. In practice, metformin is found to attenuate lung cancer development either alone or synergistically with chemotherapeutic treatment, significantly reducing lung cancer risk and mortality [5–7]. However, the molecular mechanisms of these effects of metformin remains poorly understood. It is generally believed that the anti-cancer activity of metformin is mediated by AMPK activation in either LKB1-dependent or independent manner, although other molecular mechanisms aside from AMPK activation are also implied to mediate the anti-cancer effect of metformin [8–11]. Data from research emphasizing the involvement of AMPK activation in metformin’s anti-lung cancer effect also showed that AMPK knockout or sufficient knockdown did not completely relieve the inhibitory effect of metformin on lung cancer development [8,10].

We deeply reviewed current literatures regarding the molecular action of metformin and found that Kickstein et al. reported on the direct activation of PP2A by metformin treatment: metformin disrupts the interaction between the catalytic subunit of PP2A and α4 and MID1, the 2 of which function as inhibitors of PP2A catalytic activity, and this mechanism was later confirmed by Hettich et al. [12,13]. PP2A is a well-defined tumor suppressor protein in lung cancer as well as in other cancer types [14]. In lung cancer, PP2A has been found to suppress the oncogenic activity of Akt and Myc by catalyzing their serine de-phosphorylation, as summarized by Walter et al. [15]. Xin et al. reported that dephosphorylation of Bax catalyzed by PP2A increases its pro-apoptotic activity, while Shouse et al. demonstrated that tumor suppressive role of PP2A is partially p53 dependent [16]. Notably, Chen et al. reported that α4 functioning as endogenous PP2A inhibitor is frequently upregulated in breast, liver, and lung cancers, and artificial α4 overexpression granted non-transformed cells with tumor forming ability in nude mice [17]. Disruption of MID1-α4/PP2A complex by metformin treatment was also reported by Demir et al. in prostate cancer cells in vitro, in which they found that MID1 or α4 knockdown mimicked the anti-cancer effect of metformin in an AMPK-independent manner [18]. Sacco et al. demonstrated that metformin treatment increased the protein expression of PP2A catalytic and regulatory subunit as well as the holoenzyme assembly in breast cancer cells in vitro [19]. Metformin was proposed to attenuate Alzheimer or Parkinson disease-like neuropathy by reducing the phosphorylation of tau protein or α-synuclein, respectively, in a PP2A dependent manner [20–23], but recent research claimed that metformin reduced endometrial cancer development by inhibiting PP2A [24]. These previous findings led us to the hypothesis that PP2A activation is involved in the anti-cancer activity of metformin, which was tested using A549 non and H1651 human small cell lung cancer (NSCLC) cells in the present research.

Material and Methods

Cell culture, transfection, and treatment

A549 and H1651 human non-small cell lung cancer (NSCLC) cells were purchased from American Type Culture Collection (Manassas, VA, USA). Cells were preserved in liquid nitrogen after shipment and were used on passage 2 to 5. A549 and H1651 cells were cultured in RPMI-1640 medium (STEMCELL Technologies, Vancouver, Canada) supplemented with 10% FBS (STEMCELL Technologies) and 100 U/mL of penicillin/streptomycin (Thermo Fisher Scientific) in a cell incubator with 37°C, 5% CO₂ atmosphere. α4 overexpression in A549 or H1651 cells was achieved by lentiviral transfection, and A549 or H1651 cell line with stable knockdown of A/B catalytic subunit of PP2A was constructed by lentiviral transfection of targeting shRNA. Lentiviral vectors described above were constructed by Genecopoeia (Rockville, MD, USA) and were used following manufacturer’s instructions. Metformin hydrochloride (Tocris Bioscience, Bristol, UK) was pre-diluted in complete culture medium as 10x stock and preserved under –8°C before use. OA (Tocris Bioscience) was pre-diluted in DMSO as 100x stock and preserved under –20°C before use.

Cell viability, proliferation, apoptosis, and Transwell invasion assay

A549 and H1651 cell viability was assayed using CCK-8 cell counting kit (Dojindo, Kumamoto, Japan) following manufacturer’s instructions. Then, 1.5×10⁴ cells of each experimental group were equally seeded on 96-well plates and were treated as indicated for 48 hours before cell viability assay. Cell proliferation was assayed using Click-it™ EdU microplate assay kit (Thermo Fisher Scientific) following manufacturer’s instructions. Cells were treated as indicated for 48 hours before assay. Apoptosis assay was performed using TiterTACS in situ detection kit (R&D Systems, Minneapolis, MN, USA) following manufacturer’s instructions. Cells were treated as indicated for 48 hours before assay. Transwell assay was performed using Matrigel-coated Transwell inserts (with 8.0 mm pore membrane, Corning Incorporated, Corning, NY, USA). Briefly, equal amounts of cells of each group were seeded in the insert chamber with
serum-free culture medium and inserted in complete culture medium with 10% FBS. After incubation for 24 hours, cells migrated to the bottom of the chamber were stained with crystal violet and counted under microscope.

**Western blot**

Western blot was performed using homemade, reducing polyacrylamide (Bio-Rad, Hercules, CA, USA) gel. After being separated by electrophoresis and transferred onto nitrocellulose membrane (Bio-Rad), proteins of interest were blotted with primary and HRP-conjugated secondary antibodies, which
were then detected by incubation with fluorescent ECL substrate (BosterBio, Pleasanton, CA, USA) and x-ray film (MBL International, Woburn, MA, USA). Protein expression was semi-quantified by comparing the gray scale of band of each protein visualized on x-ray film to that of the housekeeping protein β-actin processed under same conditions. Gray scale analysis was performed using ImageJ software. Primary antibodies used for western blot are as follows: PCNA (orb214367, rabbit polyclonal, Biorbyt, San Francisco, CA, USA), caspase-3 (orb153764, rabbit polyclonal, Biorbyt), active caspase-3 (ab2324, rabbit polyclonal, Abcam), Bax (NBP1-28566, mouse monoclonal, Novus Biologicals, Littleton, CO, USA); phospho-Bax (Ser184, PA5-39778, rabbit polyclonal, Thermo Fisher Scientific); Bcl-2 (658702, mouse monoclonal, BioLegend); c-Myc (sc-40, mouse monoclonal, Santa Cruz Biotechnology, Santa Cruz, CA, USA); phospho-c-Myc (Ser62, 13748, rabbit monoclonal, Cell Signaling Technology, Danvers, MA, USA); Akt (NBP2-44110, mouse monoclonal, Novus Biologicals); phosphor-Akt (Ser473, NB100-56749, mouse monoclonal, Novus Biologicals); β-actin (ab20272, mouse monoclonal, Abcam). HRP-conjugated secondary antibodies used for western blot are as follows: Rabbit Anti-Mouse IgG H&L (ab6728, Abcam); Goat Anti-Rabbit IgG H&L (ab6721, Abcam)

Statistical analysis

Statistical analysis was performed using SPSS Software (ver. 17). Each data represents the results of 6 independent replicate experiments and was presented as mean ± SDS unless otherwise indicated. All data were presented as fold induction when applicable. One-way analysis of variance (ANOVA) with Tukey’s post hoc test was used for significance test. A P value <0.05 was considered significant.

Results

Anti-cancer effect of metformin is partially dependent on the catalytic activity of PP2A

To examine our hypothesis that activation of PP2A is involved in the anti-cancer activity of metformin treatment, we employed OA for inhibiting the catalytic activity of PP2A in A549 and H1651 human NSCLC cells [12,25]. We also constructed α4 overexpression (O/E α4) or double knockdown of PP2A catalytic subunit A and B (sh-PP2Ac) to reduce PP2A activity in the
Figure 2. Metformin treatment inhibited A549 and H1651 cell invasion capacity in vitro partially in a PP2A catalytic activity-dependent manner. (A–D) Metformin dose-dependently reduced Transwell invasion of A549 and H1651 cells in vitro, which was partially attenuated by okadaic acid treatment (OA), α4 overexpression (O/E α4) or knockdown of PP2A catalytic subunit A and B (sh-PP2Ac). C and D are representative results of Transwell assay in A and B, respectively. Data in A and B was normalized to the mean value of NC group in the same panel. ns – non-significant; * P<0.05; ** P<0.01; *** P<0.001; **** P<0.0001.

2 cell lines [17]. Significant α4 overexpression and sufficient inhibition of PP2A catalytic subunit A and B expression were verified by RT-qPCR and western blot (data not shown). Cell functional assay results showed that treatment with metformin significantly reduced A549 and H1651 cell viability while increasing apoptosis in vitro in a dose dependent manner, leaving the cell proliferation status intact, and these effects could be partially reversed by combined treatment with OA, α4 overexpression or PP2Ac knockdown (Figure 1A–1F). Cell proliferation and apoptosis in A549 and H1651 cells after treatment in Figure 1C–1F were further verified by western blot detecting the protein expression level of cell proliferation and apoptosis marker genes PCNA and caspase-3 (Figure 1G). Transwell invasion assay using Matrigel-coated Transwell chamber demonstrate that treatment with metformin at 10 mM significantly reduced invasive capacity of A549 or H1651 cells by nearly 50% in vitro, which could be partially reversed by OA co-treatment at 50 nM, α4 overexpression or PP2Ac knockdown (Figure 2).
Among these results, knockdown of PP2A catalytic domain A and B showed the most significant interference on the inhibitory effect of metformin treatment on cell viability, survival and invasion in vitro. These data suggested that the anti-cancer effect of metformin is partially depended on the catalytic activity of PP2A.

**Metformin treatment increased PP2A catalytic activity**

PP2A has been demonstrated to suppress lung cancer cell growth and tumor development by decreasing the signal transduction of key oncogenic signaling pathways, including PI3K-Akt and Myc, as well as by increasing the pro-apoptotic activity of Bax via its serine phosphatase activity [15,25]. We therefore investigated whether metformin treatment would influence the serine phosphorylation level of Bax, Myc, and Akt on PP2A sensitive site in A549 cells (Figure 3). Western blot assays revealed that metformin treatment significantly increased Bax protein expression level and decreased its Ser184 phosphorylation level, resulting in a dramatic decrease in p-Bax/Bax ratio (Figure 3A–3C, 3E); metformin treatment also significantly decreased Bcl-2 protein expression level in A549 cells, possibly due to the increase in cell apoptosis (Figure 3D, 3E). Metformin treatment significantly decreased Ser62 phosphorylation of Myc protein and S473 phosphorylation of Akt protein that are known to be the target site of PP2A [15] (Figure 3F–3L). Notably,
metformin treatment showed no significant influence on Akt protein expression level but significantly reduced that of Myc protein, probably due to the ubiquitination-mediated degradation of Myc protein after loss of Ser62 phosphorylation catalyzed by PP2A [26] (Figure 3G, 3J). Our western blot assay further revealed that these influences of metformin treatment on protein expression and serine phosphorylation of Bax, Bcl-2, Myc, and Akt proteins can be significantly reversed by OA co-treatment in a dose dependent manner, α4 overexpression or PP2Ac knockdown (Figure 3). These data clearly demonstrated that metformin treatment could decrease the serine phosphorylation of PP2A substrate proteins, which could be partially...
reversed by inhibition of PP2A catalytic activity through various means, suggesting that metformin could activate PP2A in lung cancer cells in vitro.

Metformin treatment reduced tumor growth in vivo

We next investigated whether the anti-cancer effect of metformin in vivo is also mediated at least in part by PP2A. We constructed xenograft mouse model using A549 cells that were wild type (WT), α4 overexpression (O/E α4) or PP2Ac knockdown...
Figure 4. Metformin treatment inhibited tumor formation capacity of A549 in xenograft model partially in a PP2A catalytic activity-dependent manner. (A) Tumor growth curve of each group. (B, C) Tumor mass at endpoint of the tumor formation assay. (D–I), western blot detecting indicated protein expression level or serine phosphorylation level in xenografted tumor tissue. (F) Representative western blot result. Data in (B) presented as mean ±SD represents 3 independent replicate experiments. Data in (B, D, E, G–I) was normalized to the mean value of WT group in the same panel. * \( P<0.05; \) ** \( P<0.01; \) *** \( P<0.001; \) **** \( P<0.0001. \) WT, wild type.

Discussions

The anti-cancer activity of metformin treatment in various types of cancers has been acknowledged in recent years. The molecular mechanisms of these effect, however, remains to be further elucidated. A better understanding of the molecular mechanism of action of metformin might aid the development of novel therapeutic strategy for cancer management. Recognition of AMPK activation by metformin involved in its anti-cancer effect dawned in 2007 when Buzzai et al. reported the selective inhibition of metformin treatment on p53 deficient liver cancer cells but not wild type counterparts [27]. In lung cancer cells, metformin-induced AMPK activation has been shown to inhibit lung cancer cell growth in vitro and in vivo either alone or in synergy with other anti-cancer therapy [5,10,28]. However, after carefully examining the data presented by current reports, we believe that the anti-cancer effect of metformin on lung cancer cannot be explained solely by AMPK activation.
Analogously, Lin et al. reported that the anti-cancer and cisplatin-sensitizing effect of metformin treatment in NSCLC cells were partially mediated by an AMPK-independent STAT3 dephosphorylation mechanism [11].

During our literature research, we noticed that metformin treatment could directly interfere with the association of PP2A catalytic subunit, α4, and MID1, thus liberating the catalytic activity of PP2A [12,13]. PP2A has long been confirmed as a tumor suppressor in lung, breast, and ovarian cancers, although PP2A inhibition was also suggested to induce mitotic catastrophe and cell death in senescent cancer cells [14,29,30]. Our present study aimed to test whether PP2A activation was involved in the anti-cancer effect of metformin treatment on lung cancer. Our data showed that decreasing the PP2A catalytic activity either by OA treatment, α4 overexpression or stable knockdown of PP2A, catalytic subunit A and B significantly attenuated the inhibition of NSCLC cell growth or invasive capacity in vitro by metformin treatment, and PP2Ac knockdown most significantly obstructed metformin’s effect. Tumor formation assay also confirmed that α4 overexpression or PP2Ac knockdown desensitized A549 cells to metformin treatment-induced inhibition of tumor growth in vivo. Western blot assay results further demonstrated that metformin treatment lowered Ser473 phosphorylation of Akt that would result in its inactivation, Ser62 phosphorylation of Myc that would lead to its degradation, as well as Ser184 phosphorylation of Bax known to increasing its pro-apoptotic activation, and these effects were significantly weakened by OA treatment, α4 overexpression, or PP2Ac knockdown [15,25]. Collectively, our results suggested that PP2A activation by metformin is involved in its anti-cancer effect on lung cancer. Re-activation of PP2A by metformin or other targeted approach might aid lung cancer treatment.

Interestingly, our in vitro experimental data suggested that metformin treatment reduced lung cancer cell growth in vitro primarily by inducing apoptosis, considering that the cell viability assay we performed evaluated the number of living cells from the same amount of initial seeding cells; However, by western blot assay detecting cell proliferation marker PCNA and apoptosis marker caspase-3 activation, we found that cell proliferation in xenografted tumor was reduced by metformin treatment, and caspase-3 activation level was not significantly altered (data not shown). We inferred from these data that metformin might exhibit additional effect that reduced cancer cell proliferation in vivo, which warranted further investigation. Various research employed cell viability assay to evaluate the cell proliferation-inhibiting effect of metformin on lung cancer cells [31,32], but this technique is questionable, because cell viability assays reflects the change in viable cell number, which could be the overall effect of cell proliferation plus cell apoptosis. It should also be noted that sufficient knockdown of PP2A catalytic subunits or inhibitor treatment did not completely block the anti-cancer effect of metformin treatment in other experimental conditions. Although activation of PP2A by metformin has been suggested as AMPK-independent, possibility remains that activation of AMPK and PP2A might synergize to inhibit tumor development. Besides AMPK or PP2A activation, it has also been suggested that metformin inhibits lung cancer development through other mechanisms. For instance, Jin et al. demonstrated that metformin treatment reduced the mRNA and protein expression of E2F8, a presumed oncogenic transcription factor in lung cancer that functions by promoting cell cycle progression [33]. Yu et al. demonstrated that metformin treatment upregulated Sirtuin 1 to reduce acetylation of NF-E2-related factor 2, thus reducing its transcriptional activity and sensitizing lung cancer cells to epigallocatechin-3-gallate induced apoptosis [34]. Whether these phenomena are direct effects of metformin or indirect through AMPK or PP2A activation needs to be further elucidated. Previous research also pointed to the synergy effect of metformin and chemo- or targeted therapy, especially EGFR tyrosine kinase inhibitor [35,36]; whether PP2A is involved in these synergic effects should also be further investigated.

Conclusions

In the present study, our data clearly demonstrated that metformin reduced lung cancer cell growth and invasion in vitro as well as tumor development in vivo partially by activating PP2A, which inhibited the activation of tumor promoting proteins such as Bax, Myc, and Akt by catalyzing their dephosphorylation.

Conflict of interest

None.

References:

1. Hirsch FR, Scagliotti GV, Mulshine JL et al: Lung cancer: Current therapies and new targeted treatments. Lancet, 2017; 389: 299–311
2. Rosell R, Karachaliou N: Lung cancer in 2014: Optimizing lung cancer treatment approaches. Nat Rev Clin Oncol, 2015; 12: 75–76
3. Levy A, Doyen J: Metformin for non-small cell lung cancer patients: Opportunities and pitfalls. Crit Rev Oncol Hematol, 2018; 125: 41–47
4. Pernicova I, Korbonits M: Metformin – mode of action and clinical implications for diabetes and cancer. Nat Rev Endocrinol, 2014; 10: 143–56
5. Troncone M, Cargnelli SM, Villani LA et al: Targeting metabolism and AMP-activated kinase with metformin to sensitize non-small cell lung cancer (NSCLC) to cytotoxic therapy: Translational biology and rationale for current clinical trials. Oncotarget, 2017; 8: 57733–54
6. Zhong S, Wu Y, Yan X et al: Metformin use and survival of lung cancer patients: Meta-analysis findings. Indian J Cancer, 2017; 54: 63–67
7. Morgillo F, Sasso FC, De La Corte CM et al: Metformin in lung cancer: Rationale for a combination therapy. Expert Opin Investig Drugs, 2013; 22: 1401–9
8. Guo Q, Liu Z, Jiang L et al: Metformin inhibits growth of human non-small cell lung cancer cells via liver kinase B1-independent activation of adenosine monophosphate-activated protein kinase. Mol Med Rep, 2016; 13: 2590–96
9. Xiao X, He Q, Lu C et al: Metformin impairs the growth of liver kinase B1-intact cervical cancer cells. Gynecol Oncol, 2012; 127: 249–55
10. Storozhuk Y, Hopmans SN, Sanli T et al: Metformin inhibits growth and enhances radiation response of non-small cell lung cancer (NSCLC) through STAT3 and AMPK. Br J Cancer, 2013; 108: 2021–32
11. Lin CC, Yeh HH, Huang WL et al: Metformin enhances cisplatin cytotoxicity by suppressing signal transducer and activator of transcription-3 activity independently of the liver kinase B1-AMP-activated protein kinase pathway. Am J Respir Cell Mol Biol, 2013; 49: 241–50
12. Kickstein E, Krauss S, Thornhill P et al: Biguanide metformin acts on tau phosphorylation via mTOR/protein phosphatase 2A (PP2A) signaling. Proc Natl Acad Sci USA, 2010; 107: 21830–35
13. Hettich MM, Matthes F, Ryan DP et al: The anti-diabetic drug metformin reduces BACE1 protein level by interfering with the MID1 complex. PLoS One, 2014; 9: e102420
14. Sangodkar J, Farrington CC, McClintch K et al: All roads lead to PP2A: Exploiting the therapeutic potential of this phosphatase. FEBS J, 2016; 283: 1004–24
15. Walter G, Ruediger R: Mouse model for probing tumor suppressor activity of protein phosphatase 2A in diverse signaling pathways. Cell Cycle, 2012; 11: 451–59
16. Shouse GP, Nobumori Y, Liu X: A B56gamma mutation in lung cancer disrupts the p53-dependent tumor-suppressor function of protein phosphatase 2A. Oncogene, 2010; 29: 3933–41
17. Chen LP, Lai YD, Li DC et al: alpha4 is highly expressed in carcinogen-transformed human cells and primary human cancers. Oncogene, 2011; 30: 2943–53
18. Demir U, Koehler A, Schneider R et al: Metformin tumor-suppressor effect via disruption of the MID1 transialational regulator complex and AR down-regulation in prostate cancer cells. BMC Cancer, 2014; 14: 52
19. Sacco F, Silvestri A, Posca D et al: Deep proteomics of breast cancer cells reveals that metformin rewire signaling networks away from a pro-growth state. Cell Syst, 2016; 2: 159–71
20. Li J, Deng J, Sheng W et al: Metformin attenuates Alzheimer’s disease-like neuropathology in obese, leptin-resistant mice. Pharmaco Blueprint Behav, 2012; 101: 564–74
21. Matthes F, Hettich MM, Schilling J et al: Inhibition of the MID1 protein complex: A novel approach targeting APP protein synthesis. Cell Death Disov, 2018; 4: 4
22. Katila N, Bhurteil S, Shadfar S et al: Metformin lowers α-synuclein phosphorylation and upregulates neurotrophic factor in the MPTP mouse model of Parkinson’s disease. Neuropathology, 2017; 125: 396–407
23. Pérez-Revuelta BL, Hettich MM, Ciociaro A et al: Metformin lowers Ser-129 phosphorylated α-synuclein levels via mTOR-dependent protein phosphatase 2A activation. Cell Death Dis, 2014; 5: e1209
24. Hanawa S, Mitsuhashi A, Shouz M: Antitumor effects of metformin via indirect inhibition of protein phosphatase 2A in patients with endometrial cancer. PLoS One, 2018; 13: e0192759
25. Xin M, Deng X: Protein phosphatase 2A enhances the proapoptotic function of Bax through dephosphorylation. J Biol Chem, 2006; 281: 18859–67
26. Jozwik P, Forma E, Brys M, Krzeslak A: O-GlcNAcylation and metabolic reprogramming in cancer. Front Endocrinol (Lausanne), 2014; 5: 145
27. Buzzi M, Jones RG, Amaravadi RK et al: Systemic treatment with the anti-diabetic drug metformin selectively impairs p53-deficient tumor cell growth. Cancer Res, 2007; 67: 6745–52
28. Groenendijk FH, Mellaena WW, van der Burg E et al: Sorafenib synergizes with metformin in NSCLC through AMPK pathway activation. Int J Cancer, 2015; 136: 1434–44
29. Mummby M: PP2A: Unveiling a reluctant tumor suppressor. Cell, 2007; 130: 21–24
30. Hong CS, Ho W, Zhang C et al: LB100, a small molecule inhibitor of PP2A with potent chemo- and radio-sensitizing potential. Cancer Biol Ther, 2015; 16: 821–33
31. Zhang Y, Peng X, Li T et al: Metformin synergistic pemetrexed suppresses non-small-cell lung cancer cell proliferation and invasion in vitro. Cancer Med, 2017; 6: 1965–75
32. Shimazu K, Tada Y, Morinaga T et al: Metformin produces growth inhibitory effects in combination with nutlin-3a on malignant mesothelioma through a cross-talk between mTOR and p53 pathways. BMC Cancer, 2017; 17: 309
33. Jin DH, Kim Y, Lee BB et al: Metformin induces cell cycle arrest at the G1 phase through E2F8 suppression in lung cancer cells. Oncotarget, 2017; 8: 21059–20
34. Yu C, Jiao Y, Xue J et al: Metformin sensitizes non-small cell lung cancer cells to an Epigallocatechin-3-Gallate (EGCG) treatment by suppressing the Nrf2/HO-1 signaling pathway. Int J Oncol, 2017; 41: 2590–96
35. Wang X, Chen K, Yu Y et al: Metformin sensitizes lung cancer cells to an epigallocatechin-3-gallate (EGCG) treatment by suppressing the Nrf2/HO-1 signaling pathway. Int J Oncol, 2017; 136: 1434–44
36. Chen H, Yao W, Chu Q et al: Synergistic effects of metformin in combination with EGFR-TKI in the treatment of patients with advanced non-small cell lung cancer and type 2 diabetes. Cancer Lett, 2015; 369: 97–102