Metformin reduces glycometabolism of papillary thyroid carcinoma *in vitro* and *in vivo*

Chen-Tian Shen^{1,2,*}, Wei-Jun Wei^{1,*}, Zhong-Ling Qiu¹, Hong-Jun Song¹, Xin-Yun Zhang¹, Zhen-Kui Sun¹ and Quan-Yong Luo¹

¹Department of Nuclear Medicine, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, People's Republic of China

²Shanghai Jiao Tong University School of Medicine, Shanghai, People's Republic of China

*(C-T Shen and W-J Wei contributed equally to this work)

Correspondence should be addressed to Q-Y Luo

Email
lqyn@sh163.net

Abstract

More aggressive thyroid cancer cells show a higher activity of glycometabolism. Targeting cancer cell metabolism has emerged as a novel approach to prevent or treat malignant tumors. Glucose metabolism regulation effect of metformin in papillary thyroid cancer was investigated in the current study. Human papillary thyroid carcinoma (PTC) cell lines BCPAP and KTC1 were used. Cell viability was detected by CCK8 assay. Glucose uptake and relative gene expression were measured in metformin (0–10 mM for 48 h)-treated cells by ¹⁸F-FDG uptake assay and western blotting analysis, respectively. MicroPET/CT imaging was performed to detect ¹⁸F-FDG uptake *in vivo*. After treatment with metformin at 0, 2.5, 5 and 10 mM for 48 h, the ratio of p-AMPK to total AMPK showed significant rising in a dose-dependent manner in both BCPAP and KTC1, whereas p-AKT and p-mTOR expression level were downregulated. ¹⁸F-FDG uptake reduced after metformin treatment in a dose-dependent manner, corresponding to the reduced expression level of HK2 and GLUT1 *in vitro*. Xenograft model of PTC using BCPAP cells was achieved successfully. MicroPET/CT imaging showed that *in vivo* ¹⁸F-FDG uptake decreased after treatment with metformin. Immunohistochemistry staining further confirmed the reduction of HK2 and GLUT1 expression in the tumor tissue of metformin-treated PTC xenograft model. In conclusion, metformin could reduce glucose metabolism of PTC *in vitro* and *in vivo*. Metformin, by targeting glycometabolism of cancer cells, could be a promising adjuvant therapy alternative in the treatment modality of advanced thyroid carcinoma.

Key Words

- ▶ metformin
- ▶ glucose metabolism
- ▶ papillary thyroid carcinoma
- ▶ ¹⁸F-FDG uptake

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Introduction

Thyroid cancer is increasing globally, especially in young women and differentiated thyroid cancer (DTC) is the most predominant type (Chen *et al.* 2016, Siegel *et al.* 2016). Clinically, it has been widely accepted that ¹⁸F-fluoro-2-deoxy-D-glucose (¹⁸F-FDG) uptake in metastatic lesions

of DTC could provide valuable prognostic information. Metastatic lesions with high ¹⁸F-FDG uptake have more dedifferentiated, aggressive and metabolically active tumor cells, thereby indicating a poor prognosis. Conversely, a negative ¹⁸F-FDG PET/CT imaging predicts

a more favorable prognosis even in patients with positive radioiodine whole body scan (Masson-Deshayes *et al.* 2015, Pryma *et al.* 2006, Salvatori *et al.* 2015). In molecular level, overexpression of glucose transporter-1 (GLUT1) on the cell membrane of thyroid cancer could contribute to this phenomenon (Schonberger *et al.* 2002).

Metformin (N,N-dimethylbiguanide), an oral biguanide drug with well-established efficacy and safety profiles, is commonly prescribed as the first-line treatment for patients with type 2 diabetes mellitus (T2DM). Generally, this drug improves blood glucose control and insulin sensitivity by reducing hepatic glucose production, gluconeogenesis and intestinal glucose absorption as well as increasing peripheral glucose uptake (Crandall *et al.* 2008). Recently, its anti-tumor activity has been widely investigated. There are more than 250 registered clinical trials in patients with breast, ovarian, lung, colorectal and pancreatic cancers using metformin alone or in combination with other therapies (ClinicalTrials.gov). Although popularly studied, the exact effect of metformin on thyroid cancer is still controversial.

Experimental studies have demonstrated that metformin could inhibit cell proliferation and promote apoptosis in multiple thyroid cancer cell lines through adenosine monophosphate-activated protein kinase (AMPK) activation, mammalian target of rapamycin (mTOR) signaling inhibition and cell cycle arrest (Chen *et al.* 2012, Cho *et al.* 2014a,b, Hanly *et al.* 2015). Epidemiological studies have showed that the use of metformin was linked to a decreased risk of thyroid cancer in T2DM patients and a higher remission rate in T2DM patients with thyroid cancer (Klubo-Gwiedzinska *et al.* 2013, Tseng 2014). However, meta-analyses have indicated that metformin-induced reduction of cancer incidence and mortality seemed to be of modest magnitude and not affecting all populations equally (Gandini *et al.* 2014). And more recently, an observational study demonstrated that neither use of metformin nor of any other antidiabetic drugs was associated with a decreased risk of thyroid cancer (Becker *et al.* 2015). However, the total number of metformin-used thyroid cancer patients in this study was much small.

Targeting cancer cell metabolism has emerged as a novel approach to prevent or treat cancers and one of the primary metabolic changes observed in malignant cell transformation is an increased catabolic glucose metabolism (Pollak 2012, Vander Heiden 2011). Anticancer effects of metformin in proliferation and apoptosis of thyroid cancer have been frequently studied. However, few studies have reported the effect of metformin on

the glucose metabolism in thyroid cancer. Based on this, we focused on the glycometabolism regulation effect of metformin in thyroid cancer *in vitro* and *in vivo* in the current study.

Materials and methods

Cell culture

Human papillary thyroid carcinoma cell lines BCPAP (DSMZ, German Collection of Microorganisms and Cell Cultures) and KTC1 (kindly provided by Stem Cell Bank, Chinese Academy of Sciences, Shanghai, China) were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin and 1% nonessential amino acids (100×) (Gibco). All the cells were incubated at 37°C in a humidified chamber supplemented with 5% CO₂.

Cell viability assay

Cells (1 × 10⁴ cells/well) were seeded in 96-well plates. After culturing for 24 h, cells were rinsed with phosphate-buffered saline (PBS) and treated with metformin (MP Biomedicals, Santa Ana, CA, USA) at various concentrations (0–50 mM) for 48 h. Cell viability was then evaluated using a CCK8 (Cell Counting kit-8) kit according to the manufacturer's protocol. Briefly, 10 µL CCK8 solution (YEASEN, Shanghai, China) was added to each well, and the samples were incubated at 37°C for 2 h before the absorbance was measured at 450 nm wave length. Each experimental condition, including blank wells, control wells and wells treated with metformin, was assayed in duplicate, and all experiments were performed at least three times.

In vitro ¹⁸F-FDG uptake assay

For ¹⁸F-FDG uptake measurement, cells were seeded in 6-well plates (5 × 10⁵ cells per well) overnight, and then subsequently treated with metformin at 0, 2.5, 5 and 10 mM for 48 h. Later, the cell culture medium was changed by sugar-free RPMI-1640 culture medium. After 6 h, cells were incubated with ¹⁸F-FDG (Atomic firm Sinovac Pharmaceutical Co., LTD., Shanghai, China) at a final concentration of 37 kBq/mL. After 60-min incubation, cells were washed twice with cold phosphate-buffered saline (PBS) and lysed with 0.1 M NaOH. Radioactivity was measured using a gamma counter (Capintec, Inc., USA) and expressed as % uptake per well relative to controls. Experiments were repeated for three times.

Western blotting analysis

Cells were seeded in 6-well plates at a density of 1×10^6 cells/well. After relevant treatment, cells were harvested in RIPA lysis buffer containing proteinase and phosphatase inhibitors (Beyotime, Nantong, Jiangsu, China). Protein was quantified using a BCA protein assay kit (Beyotime). Equal amounts of cell lysates were separated by 6–10% SDS-PAGE and electrophoretically transferred to PVDF membrane. The membrane was then blocked and probed with primary antibodies (anti-AMPK, anti-phospho-AMPK, anti-GLUT1, anti-HK2, anti-AKT, anti-phospho-AKT, anti-mTOR, anti-phospho-mTOR and anti- β -actin; 1:1000) followed by HRP (horseradish peroxidase)-labeled goat anti-mouse IgG or HRP-labeled goat anti-rabbit IgG (1:5000). Chemiluminescence was used to analyze protein levels, and β -actin was used as a protein loading control. All antibodies used in this study were purchased from Proteintech Group, Inc., Rosemont, IL, USA. Semi-quantitative analysis was conducted using ImageJ 1.49v (NIH, Bethesda, MD, USA). Experiments were repeated for three times.

Immunohistochemistry (IHC)

Paraffin-embedded tissues from xenograft tumor were cut into $3 \mu\text{m}$ sections. Endogenous peroxidase activity was blocked with 3% H_2O_2 for 15 min. Antigen retrieval was performed by boiling the sections for 10 min in citrate buffer (pH 6.0) and cooling at room temperature, followed by blocking with 10% normal goat serum for 1 h. After incubation with primary antibody (anti-GLUT1 and anti-HK2; 1:100) overnight at 4°C , the sections were incubated with horseradish peroxidase- (HRP-) conjugated anti-mouse/rat secondary antibodies. After washing with PBS three times for 5.0 min each time, peroxidase substrate DAB kit was used for visualization according to the manufacturer's instructions. Semi-quantitative analysis was performed by digital image analysis with the Image-Pro Plus 6.0 software (Media Cybernetics, Rockville, MD, USA).

Xenograft model

Animal studies were carried out in compliance with the guideline on Administration of Lab Animals (Ministry of Science and Technology of China) and approved by the Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital. For PTC xenograft implantation, a total of 2×10^7 cells were subcutaneously injected into the left forelimb of 6-week-old female

BALB/c-nu mice. The animals were kept in individual ventilated cages in compliance with institutional guidelines.

MicroPET/CT imaging and analysis

MicroPET/CT scans and image analysis were performed using a Super Nova MicroPET/CT (Pingseng Healthcare (KunShan), Inc., Kunshan, Jiangsu, China). Water and food were not supplied until ^{18}F -FDG injection at least for 8 h. Each tumor-bearing mouse was injected with 4.959–4.958 MBq ($107\text{--}134 \mu\text{Ci}$) of ^{18}F -FDG (Atomic firm Sinovac Pharmaceutical Co., LTD., Shanghai) in $200 \mu\text{L}$ of saline via tail vein. Twelve-minute static scans were acquired at 1 h after injection, and animals were maintained under 2% isoflurane anesthesia during scanning period. Body temperature was maintained using a heating pad provided with the small-animal PET/CT system. For semi-quantitative analysis, 3-dimensional regions of interest (ROIs) were carefully delineated over the borders of the tumor (T) and a part of the liver as a non-target (NT) reference. ^{18}F -FDG uptake was acquired as SUVmax automatically after delineating the ROIs. The SUVmax ratio (T/NT) was calculated and compared between groups.

Statistics analysis

Analyses were performed using the Statistical Package for the Social Sciences, version 20.0 (SPSS) and GraphPad prism version 5.0 (GraphPad Software). All group data are expressed as means $\pm$ standard deviation (s.d.). Student's *t*-test was performed to compare means of different groups. All *P* values are two-tailed. $P < 0.05$ was considered as a statistically significant difference.

Results

Cell viability inhibition *in vitro*

To determine the effect of metformin on the viability of thyroid cancer cell, we performed CCK8 assay. Two PTC cell lines, KTC1 and BCPAP, were treated with metformin at various concentrations (0, 10, 20, 30, 40 and 50 mM) for 48 h. The results demonstrated that after treatment with metformin, all thyroid cancer cell lines showed dose-dependent decreases in cell viabilities (Fig. 1).

AMPK activation

To confirm metformin-mediated AMPK activation through AMPK phosphorylation in PTC cell lines, proteins from

Figure 1

Effects of metformin on cell viability in human papillary thyroid cancer cells. * $P < 0.05$.

metformin-treated cells (KTC1 and BCPAP) at 0, 2.5, 5 and 10mM for 48h were harvested and the expression of AMPK and p-AMPK was measured by western blotting. As can be seen in Fig. 2, the ratio of p-AMPK to total AMPK showed a significant rise in a dose-dependent manner in both KTC1 and BCPAP.

^{18}F -FDG uptake *in vitro*

^{18}F -FDG enters cell via the same facilitative transporters of glucose. It is then phosphorylated and trapped in the cytoplasm. So glucose consumption can be detected and visualized non-invasively by using ^{18}F -FDG. As showed in Fig. 3(A and B), after treatment with metformin at 0, 2.5,

5 and 10mM for 48h, PTC cells showed a dose-dependent reduction of ^{18}F -FDG uptake.

Decreased expression of GLUT1 and HK2 *in vitro*

Glucose transporter-1 (GLUT1) and hexokinase-2 (HK2) are among the major proteins regulating transportation and transformation of glucose, respectively. Their expression level and function status are of great significance in the glucose metabolism in cancer cells (Colell *et al.* 2007, Mondal *et al.* 2015). Proteins from metformin-treated cells (KTC1 and BCPAP) at 0, 2.5, 5 and 10mM for 48h were harvested, and the expression of HK2 and GLUT1 was measured by western blotting. The results demonstrated that HK2 and GLUT1 in the two PTC cell lines showed dose-dependent decreases (Fig. 3C, D, E and F).

AKT/mTOR signaling suppression

AKT/mTOR pathway is an essential signaling cascade that interacts with glucose metabolism in cancer cells (Robey & Hay 2009). Proteins from metformin (0, 2.5, 5 and 10mM for 48h) -treated PTC cells (KTC1 and BCPAP) were harvested, and the expression level of AKT, p-AKT, mTOR and p-mTOR was measured by western blotting. The results demonstrated that after treatment with metformin, p-AKT and p-mTOR were significantly decreased in PTC cell lines (Fig. 4).

In vivo ^{18}F -FDG microPET/CT imaging

Based on the above evidence, ^{18}F -FDG microPET/CT imaging was performed to further confirm the effects of metformin on glucose metabolism of PTC *in vivo*. Xenograft model of PTC using BCPAP cells was successfully achieved. After 2 weeks, two groups (A and B) were randomly divided with 6 mice per group. Metformin (300mg/kg, IP, QD) treatment was done for one week in group A (metformin/+), whereas same volume of saline was intraperitoneally injected as control in group B (metformin/-). After three weeks of cells injection, *in vivo* ^{18}F -FDG microPET/CT imaging was performed. The results showed that, in accordance with the results of *in vitro* ^{18}F -FDG uptake assay, after treatment with metformin, the SUVmax ratio of T/NT was much lower in group A compared to that in group B (Figs 5 and 6A).

Decreased expression of GLUT1 and HK2 *in vivo*

After microPET/CT scan, mice were killed, and the expression of HK2 and GLUT1 was detected by

Figure 2

Western blotting analysis of AMPK and p-AMPK in metformin-treated cells at different concentrations. (A) KTC1; (C) BCPAP; (B) and (D), semi-quantitative analysis of blots in (A) and (C), respectively. * $P < 0.05$.

Figure 3

In vitro ¹⁸F-FDG uptake assay and western blotting analysis of HK2 and GLUT1 in metformin-treated cells at different concentrations. (A) and (C), KTC1; (B) and (E), BCPAP; (D) and (F), semi-quantitative analysis of blots in (C) and (E), respectively. **P* < 0.05.

immunohistochemistry. Brown staining was located in the cytoplasm and membrane of BCPAP cell. The intensity of staining was much lower in metformin-treated group compared to that in non-metformin-treated group. These results indicated that the expression of HK2 and GLUT1 was decreased after metformin treatment *in vivo* (Figs 7 and 6B).

Discussion

In the current study, we found that metformin, as the most widely prescribed oral hypoglycemic agent in T2DM patients, could downregulate the expression of GLUT1 and HK2, thus decreasing the rate of glucose metabolism in papillary thyroid carcinoma *in vitro* and *in vivo*.

The anticancer effects of metformin have been widely studied recently, whereas the underlying mechanism is still not clearly illuminated. Mainly, the potential mechanisms involved are divided into two ways: 'direct effect' and 'indirect effect'

(Koritzinsky 2015, Luengo *et al.* 2014). The direct anticancer effects of metformin are found to be mainly mediated by AMPK-dependent way as well as AMPK-independent process. For AMPK-dependent way, studies have demonstrated that metformin's primary cellular targets are the mitochondrial complex I (Owen *et al.* 2000, Wheaton *et al.* 2014) and the mitochondrial glycerophosphate dehydrogenase (mGPD) enzyme (Madiraju *et al.* 2014). AMPK is activated due to the decrease of oxygen consumption and adenosine triphosphate (ATP) production, which results from the inhibition of complex I and consequently inhibition of the mitochondrial electron transport. This effect induces cell cycle arrest and inhibits protein synthesis in cancer cells (Hawley *et al.* 2010, Steinberg & Kemp 2009). Cho and coworkers investigated the therapeutic potential of metformin in PTC, and their results indicated that metformin had anti-tumorigenic effects in PTC by activation of AMPK signaling and inhibition of AKT signaling (Cho *et al.* 2014a,b). However, other

Figure 4

Western blotting analysis of AKT, p-AKT, mTOR and p-mTOR in metformin-treated cells at different concentrations. (A) KTC1; (C) BCPAP; (B) and (D), semi-quantitative analysis of blots in (A) and (C), respectively. * $P < 0.05$.

studies have reported that metformin could also regulate cancer cell biology in an AMPK-independent way through the inhibition of the unfolded protein response with a consequent apoptosis, preventing angiogenesis and exerting toxicity on cancer stem cells (Kourelis & Siegel 2012). In addition, metformin-reduced circulating levels of glucose, insulin and other factors that can stimulate tumor cells' proliferation

is considered as its indirect effect (Algire *et al.* 2010, Sui *et al.* 2015).

Metabolic changes, known as the Warburg effect or aerobic glycolysis, have been discovered in the context of cancer diseases with the phenomenon that under aerobic conditions, proliferating cancer cells metabolize approximately tenfold more glucose to lactate in a given time than normal surrounding ones (Cairns *et al.* 2011,

Figure 5

^{18}F -FDG MicroPET/CT imaging in non-metformin-/metformin-treated xenograft model of PTC. A full colour version of this figure is available at <http://dx.doi.org/10.1530/JME-16-0134>.

Figure 6

(A) SUVmax ratio (T/NT) comparison between non-metformin- and metformin-treated xenograft model of PTC in ^{18}F -FDG MicroPET/CT imaging. (B) Semi-quantitative analysis of immunohistochemistry results. T, SUVmax of tumor; NT, SUVmax of nontarget reference (liver). * $P < 0.05$.

Koppenol *et al.* 2011). Recently, AMPK activation is found to have the ability to negatively regulate aerobic glycolysis (the Warburg effect) in cancer cells and suppress tumor growth (Faubert *et al.* 2013). Therefore, based on the fact that metformin is an activator of AMPK, another important role of metformin that could be involved in its anticancer activities is its capability to modulate the tumor cell metabolism.

Studies have showed that metformin could directly inhibit the enzymatic function of HK2 in different cancer models comprising Calu-1 cells as a model of non-small-cell lung cancer and in MDA-MB231 as a model of triple-negative breast cancer (Marini *et al.* 2013, Salani *et al.* 2013). In all these cells, metformin determined a

dose- and time-dependent reduction in ^{18}F -FDG uptake. However, Habibollahi *et al.* (2013) demonstrated that metformin, through activation of the AMPK pathway, exerted a dose-dependent increase in tumor glucose uptake in colon cancer cell lines HT29 (human) and MC26 (murine). In the current study, we examined the effect of metformin on the glucose metabolism in PTC cell lines, and the results demonstrated that metformin could decrease the ^{18}F -FDG uptake in BCPAP and KTC1 cells. To the best of our knowledge, this is the first study that tried to elucidate the anticancer effect of metformin under a cellular metabolism way in thyroid carcinoma. Although reduction of GLUT1 and HK2, activation of AMPK and inhibition of AKT/mTOR signaling could be of great importance during this process, the exact underlying mechanism of how activation of AMPK could regulate the expression of key enzymes of glycolytic pathway including glucose transporters and hexokinases in thyroid cancer cells is still not clearly known. This is the major limitation of our current work and intensive studies should be performed to further clarify the potential mechanism.

In conclusion, our results demonstrated that after treatment with metformin, PTC cells showed a dose-dependent reduction of ^{18}F -FDG uptake both *in vitro* and *in vivo* in accordance with the downregulated expression of key enzymes in glycolytic pathway including GLUT1 and HK2. Activation of AMPK and inhibition of AKT/mTOR signaling by metformin might play an essential role in the process of this effect. Based on these findings, metformin by targeting glucose metabolism of cancer cell could be a promising adjuvant therapy alternative in the treatment modality of advanced thyroid carcinoma.

Figure 7

Immunohistochemistry analysis of HK2 and GLUT1 expression in non-metformin- and metformin-treated PTC xenograft tissues. NC, negative control. A full colour version of this figure is available at <http://dx.doi.org/10.1530/JME-16-0134>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

Luo designed the study. Shen and Wei performed the experiments. Shen, Wei, Qiu and Song analyzed and interpreted the data. Shen wrote the manuscript. Luo, Sun and Zhang revised the manuscript. All authors read and approved the final manuscript.

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References

- Algire C, Amrein L, Zakikhani M, Panasci L & Pollak M 2010 Metformin blocks the stimulative effect of a high-energy diet on colon carcinoma growth *in vivo* and is associated with reduced expression of fatty acid synthase. *Endocrine-Related Cancer* **17** 351–360. (doi:10.1677/ERC-09-0252)
- Becker C, Jick SS, Meier CR & Bodmer M 2015 No evidence for a decreased risk of thyroid cancer in association with use of metformin or other antidiabetic drugs: a case-control study. *BMC Cancer* **15** 719. (doi:10.1186/s12885-015-1719-6)
- Cairns RA, Harris IS & Mak TW 2011 Regulation of cancer cell metabolism. *Nature Reviews Cancer* **11** 85–95. (doi:10.1038/nrc2981)
- Chen G, Xu S, Renko K & Derwahl M 2012 Metformin inhibits growth of thyroid carcinoma cells, suppresses self-renewal of derived cancer stem cells, and potentiates the effect of chemotherapeutic agents. *Journal of Clinical Endocrinology and Metabolism* **97** E510–E520. (doi:10.1210/jc.2011-1754)
- Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ & He J 2016 Cancer statistics in China, 2015. *CA: A Cancer Journal for Clinicians* **66** 115–132. (doi:10.1017/S0009840X15002048)
- Cho SW, Yi KH, Han SK, Sun HJ, Kim YA, Oh BC, Park YJ & Park DJ 2014a Therapeutic potential of metformin in papillary thyroid cancer *in vitro* and *in vivo*. *Molecular and Cellular Endocrinology* **393** 24–29. (doi:10.1016/j.mce.2014.05.021)
- Cho SW, Yi KH, Han SK, Sun HJ, Kim YA, Oh BC, Park YJ & Park do J 2014b Therapeutic potential of metformin in papillary thyroid cancer *in vitro* and *in vivo*. *Molecular and Cellular Endocrinology* **393** 24–29. (doi:10.1016/j.mce.2014.05.021)
- Colell A, Ricci JE, Tait S, Milasta S, Maurer U, Bouchier-Hayes L, Fitzgerald P, Guio-Carrion A, Waterhouse NJ, Li CW, *et al.* 2007 GAPDH and autophagy preserve survival after apoptotic cytochrome c release in the absence of caspase activation. *Cell* **129** 983–997. (doi:10.1016/j.cell.2007.03.045)
- Crandall JP, Knowler WC, Kahn SE, Marrero D, Florez JC, Bray GA, Haffner SM, Hoskin M & Nathan DM 2008 The prevention of type 2 diabetes. *Nature Clinical Practice Endocrinology and Metabolism* **4** 382–393. (doi:10.1038/ncpendmet0843)
- Faubert B, Boily G, Izreig S, Griss T, Samborska B, Dong Z, Dupuy F, Chambers C, Fuerth BJ, Viollet B, *et al.* 2013 AMPK is a negative regulator of the Warburg effect and suppresses tumor growth *in vivo*. *Cell Metabolism* **17** 113–124. (doi:10.1016/j.cmet.2012.12.001)
- Gandini S, Puntoni M, Heckman-Stoddard BM, Dunn BK, Ford L, DeCensi A & Szabo E 2014 Metformin and cancer risk and mortality: a systematic review and meta-analysis taking into account biases and confounders. *Cancer Prevention Research* **7** 867–885. (doi:10.1158/1940-6207.CAPR-13-0424)
- Habibollahi P, van den Berg NS, Kuruppu D, Loda M & Mahmood U 2013 Metformin – an adjunct antineoplastic therapy – divergently modulates tumor metabolism and proliferation, interfering with early response prediction by ¹⁸F-FDG PET imaging. *Journal of Nuclear Medicine* **54** 252–258. (doi:10.2967/jnumed.112.107011)
- Hanly EK, Bednarczyk RB, Tuli NY, Moscatello AL, Halicka HD, Li J, Geliebter J, Darzynkiewicz Z & Tiwari RK 2015 mTOR inhibitors sensitize thyroid cancer cells to cytotoxic effect of vemurafenib. *Oncotarget* **6** 39702–39713. (doi:10.18632/oncotarget.4052)
- Hawley SA, Ross FA, Chevtzoff C, Green KA, Evans A, Fogarty S, Towler MC, Brown LJ, Ogunbayo OA, Evans AM, *et al.* 2010 Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. *Cell Metabolism* **11** 554–565. (doi:10.1016/j.cmet.2010.04.001)
- Klubo-Gwiedzdinska J, Costello J Jr, Patel A, Bauer A, Jensen K, Mete M, Burman KD, Wartofsky L & Vasko V 2013 Treatment with metformin is associated with higher remission rate in diabetic patients with thyroid cancer. *Journal of Clinical Endocrinology and Metabolism* **98** 3269–3279. (doi:10.1210/jc.2012-3799)
- Koppenol WH, Bounds PL & Dang CV 2011 Otto Warburg's contributions to current concepts of cancer metabolism. *Nature Reviews Cancer* **11** 325–337. (doi:10.1038/nrc3038)
- Koritzinsky M 2015 Metformin: a novel biological modifier of tumor response to radiation therapy. *International Journal of Radiation Oncology, Biology, Physics* **93** 454–464. (doi:10.1016/j.ijrobp.2015.06.003)
- Kourelis TV & Siegel RD 2012 Metformin and cancer: new applications for an old drug. *Medical Oncology* **29** 1314–1327. (doi:10.1007/s12032-011-9846-7)
- Luengo A, Sullivan LB & Heiden MG 2014 Understanding the complexity of metformin action: limiting mitochondrial respiration to improve cancer therapy. *BMC Biology* **12** 82. (doi:10.1186/s12915-014-0082-4)
- Madiraju AK, Erion DM, Rahimi Y, Zhang XM, Braddock DT, Albright RA, Prigaro BJ, Wood JL, Bhanot S, MacDonald MJ, *et al.* 2014 Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. *Nature* **510** 542–546. (doi:10.1038/nature13270)
- Marini C, Salani B, Massollo M, Amaro A, Esposito AI, Orenco AM, Capitanio S, Emionite L, Riondato M, Bottoni G, *et al.* 2013 Direct inhibition of hexokinase activity by metformin at least partially impairs glucose metabolism and tumor growth in experimental breast cancer. *Cell Cycle* **12** 3490–3499. (doi:10.4161/cc.26461)
- Masson-Deshayes S, Schvartz C, Dalban C, Guendouzen S, Pochart JM, Dalac A, Fieffe S, Bruna-Muraille C, Dabakuyo-Yonli TS & Papathanassiou D 2015 Prognostic value of (18)F-FDG PET/CT metabolic parameters in metastatic differentiated thyroid cancers. *Clinical Nuclear Medicine* **40** 469–475. (doi:10.1097/RLU.0000000000000780)
- Mondal S, Roy D, Camacho-Pereira J, Khurana A, Chini E, Yang L, Baddour J, Stilles K, Padmabandu S, Leung S, *et al.* 2015 HSulf-1 deficiency dictates a metabolic reprogramming of glycolysis and TCA cycle in ovarian cancer. *Oncotarget* **6** 33705–33719. (doi:10.18632/oncotarget.5605)
- Owen MR, Doran E & Halestrap AP 2000 Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the

- mitochondrial respiratory chain. *Biochemical Journal* **348** 607–614. (doi:10.1042/bj3480607)
- Pollak MN 2012 Investigating metformin for cancer prevention and treatment: the end of the beginning. *Cancer Discovery* **2** 778–790. (doi:10.1158/2159-8290.CD-12-0263)
- Pryma DA, Schoder H, Gonen M, Robbins RJ, Larson SM & Yeung HW 2006 Diagnostic accuracy and prognostic value of 18F-FDG PET in Hurthle cell thyroid cancer patients. *Journal of Nuclear Medicine* **47** 1260–1266.
- Robey RB & Hay N 2009 Is Akt the 'Warburg kinase'?-Akt-energy metabolism interactions and oncogenesis. *Seminars in Cancer Biology* **19** 25–31. (doi:10.1016/j.semcancer.2008.11.010)
- Salani B, Marini C, Rio AD, Ravera S, Massollo M, Orengo AM, Amaro A, Passalacqua M, Maffioli S, Pfeffer U, *et al.* 2013 Metformin impairs glucose consumption and survival in Calu-1 cells by direct inhibition of hexokinase-II. *Scientific Reports* **3** 2070. (doi:10.1038/srep02070)
- Salvatori M, Biondi B & Rufini V 2015 Imaging in endocrinology: 2-[18F]-fluoro-2-deoxy-d-glucose positron emission tomography/computed tomography in differentiated thyroid carcinoma: clinical indications and controversies in diagnosis and follow-up. *European Journal of Endocrinology* **173** R115–R130. (doi:10.1530/EJE-15-0066)
- Schonberger J, Ruschoff J, Grimm D, Marienhagen J, Rummele P, Meyringer R, Kossmehl P, Hofstaedter F & Eilles C 2002 Glucose transporter 1 gene expression is related to thyroid neoplasms with an unfavorable prognosis: an immunohistochemical study. *Thyroid* **12** 747–754. (doi:10.1089/105072502760339307)
- Siegel RL, Miller KD & Jemal A 2016 Cancer statistics, 2016. *CA: A Cancer Journal for Clinicians* **66** 7–30. (doi:10.1017/S0009840X15002851)
- Steinberg GR & Kemp BE 2009 AMPK in health and disease. *Physiological Reviews* **89** 1025–1078. (doi:10.1152/physrev.00011.2008)
- Sui X, Xu Y, Wang X, Han W, Pan H & Xiao M 2015 Metformin: a novel but controversial drug in cancer prevention and treatment. *Molecular Pharmaceutics* **12** 3783–3791. (doi:10.1021/acs.molpharmaceut.5b00577)
- Tseng CH 2014 Metformin reduces thyroid cancer risk in Taiwanese patients with type 2 diabetes. *PLoS ONE* **9** e109852. (doi:10.1371/journal.pone.0109852)
- Vander Heiden MG 2011 Targeting cancer metabolism: a therapeutic window opens. *Nature Reviews Drug Discovery* **10** 671–684. (doi:10.1038/nrd3504)
- Wheaton WW, Weinberg SE, Hamanaka RB, Soberanes S, Sullivan LB, Anso E, Glasauer A, Dufour E, Mutlu GM, Budigner GS, *et al.* 2014 Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis. *eLife* **3** e02242. (doi:10.7554/elife.02242)

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