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Invited review

Metformin and cancer: Between the bioenergetic disturbances and the antifolate activity

J.A. Jara [a], R. López-Muñoz [b,*]

[a] Unidad de Farmacología y Farmacogenética, ICOD, Facultad de Odontología, Universidad de Chile, Santiago, Chile
[b] Instituto de Farmacología y Morfofisiología, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Valdivia, Chile

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ABSTRACT

For decades, metformin has been the first-line drug for the treatment of type II diabetes mellitus, and it thus is the most widely prescribed antihyperglycemic drug. Retrospective studies associate the use of metformin with a reduction in cancer incidence and cancer-related death. However, despite extensive research about the molecular effects of metformin in cancer cells, its mode of action remains controversial. In this review, we summarize the current molecular evidence in an effort to elucidate metformin's mode of action against cancer cells. Some authors describe that metformin acts directly on mitochondria, inhibiting complex I and restricting the cell's ability to cope with energetic stress. Furthermore, as the drug interrupts the tricarboxylic acid cycle, metformin-induced alteration of mitochondrial function leads to a compensatory increase in lactate and glycolytic ATP. It has also been reported that cell cycle arrest, autophagy, apoptosis and cell death induction is mediated by the activation of AMPK and Redd1 proteins, thus inhibiting the mTOR pathway. Additionally, unbiased metabolomics studies have provided strong evidence to support that metformin alters the methionine and folate cycles, with a concomitant decrease in nucleotide synthesis. Indeed, purines such as thymidine or hypoxanthine restore the proliferation of tumor cells treated with metformin *in vitro*. Consequently, some authors prefer to refer to metformin as an "antimetabolite drug" rather than a "mitochondrial toxin". Finally, we also review the current controversy concerning the relationship between the experimental conditions of *in vitro*-reported effects and the plasma concentrations achieved by chronic treatment with metformin.

Contents

1. Why metformin and cancer?

Metformin, a biguanide, is an antihyperglycemic agent that has for decades been the first-line treatment for type II diabetes mellitus; indeed, it is the most widely prescribed antidiabetic drug. Despite the extensive use of metformin as an antidiabetic for 40 years, the first report indicating an anticancer effect in mammals

* Corresponding author at: Instituto de Farmacología y Morfofisiología, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Campus Isla Teja s/n., P.O. Box: 5090000, Valdivia, Chile. Tel.: +56632444321.
E-mail addresses: rodrigo.lopez@uach.cl, rodlopez@u.uchile.cl (R. López-Muñoz).

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was in 2001 [1], and the first report of a reduced risk of cancer in patients with type II diabetes treated with metformin was published only 10 years ago [2]. Since then, more than 150 articles reporting different effects of metformin in human cancers have been published. More importantly, more than 200 ongoing clinical trials, in almost all type of cancers, assessing the potential of metformin as an adjuvant or neoadjuvant chemotherapy agent or as an enhancer of classic chemotherapy are registered on www.clinicaltrials.gov. However, the mode of action explaining the antitumor and chemopreventive effects of metformin remains a matter of controversy. In this review, we present the current evidence suggesting some of the hypothesized mechanisms of action for this drug.

2. Mode of action of metformin as an antihyperglycemic drug

The first hypothesis to explain the mode of action of metformin as an antihyperglycemic drug involved the stimulation of glucose uptake by muscle [3]. There is a growing body of evidence suggesting that the primary effect of metformin as an antidiabetic is to decrease hepatic glucose production as a consequence of gluconeogenesis inhibition [4]. Furthermore, several reports indicate that metformin administration leads to a reduction in ATP levels in the liver and a decrease in the ATP/ADP ratio, resulting in a reduction in cellular bioenergetics [5,6].

At the molecular level, the most described mode of action of metformin is the inhibition of mitochondrial complex I (NADH ubiquinone oxidoreductase), the first component of the electron transport chain [7]. Complex I inhibition by metformin could interrupt mitochondrial respiration, inducing a decrease in proton-driven ATP production and causing an energetic stress and reduction in the AMP/ATP ratio. Consequently, it was shown in the last decade that AMP/ATP ratio decreases stimulates AMP-activated protein kinase (AMPK); this enzyme was therefore considered to be a major sensor and mediator of the glucose-lowering effect of metformin [8,9]. AMPK activation in muscle can also increase glucose consumption, and hepatic AMPK activation can inhibit gluconeogenesis and activate glycolysis [10]. Both of these effects of metformin may decrease blood glucose and contribute to its antihyperglycemic effect in type II diabetes.

Although AMPK was once considered the pivotal executor of metformin's glycemic action, loss of function of AMPK and the upstream kinase LKB1 in mice revealed that AMPK may not be required for the observed gluconeogenesis suppression induced by metformin [5]. In addition, an AMPK-independent mechanism has been proposed, wherein metformin may be antagonized by glucagon-dependent cyclic AMP (cAMP) signaling [11]. In this way, metformin disturbs the glucagon activation of adenylyl cyclase and consequent cAMP production, thereby inhibiting the activation of cAMP-dependent protein kinase (PKA). The activation of PKA decreases fructose-2,6,-bisphosphate levels, thus enhancing gluconeogenesis in the liver and increasing blood glucose levels [12]. Additionally, recent data show that metformin treatment alters the hepatocellular redox state by inhibiting mitochondrial glycerophosphate dehydrogenase (mGPD), an enzyme that transports cytosolic reducing equivalents from NADH to the mitochondria through the glycerol-phosphate shuttle. It is noteworthy that a decrease in reducing equivalents, as well as complex I inhibition, compromises the ability of mitochondria to provide reducing equivalents to the electron transport chain to promote the production of ATP [13].

Thus, considering the increasing evidence that mitochondrial metabolism plays a pivotal role in supporting tumor growth by delivering both ATP and metabolic intermediates that can be used in anabolic reactions [14,15], an understanding of the actions of metformin in energy metabolism, particularly mitochondrial function, is relevant within the context of the potential applications of metformin in oncology.

3. Metformin mode of action in cancer cell bioenergetics

The recognition that type II diabetes mellitus is associated with increased cancer risk has led to increased interest in the therapeutic potential of various antidiabetic drugs such as metformin [16,17]. Of the abovementioned modes of action, the first one explored was metformin's mitochondrial activity.

Efficient mitochondrial activity and complex I function have been shown to be essential for the promotion of aerobic glycolysis and the Warburg effect [18], and the induction of mitochondria-mediated apoptosis by metformin was described in glioma cells in 2007 [19]. In 2010, it was reported that metformin increases the fraction of uncoupled respiration [20], which is important because the induction of mitochondrial uncoupling itself *via* the overexpression of UPCs proteins is able to inhibit tumor growth in breast cancer cells (the MDA-MB-231 cell line) [21].

Although it has been shown that metformin inhibits mitochondrial complex I in cancer cells (Fig. 1), there is no molecular description to date of the interaction between metformin and this NADPH reductase. However, some recent evidence lends support to this hypothesis. Andrzejewski et al. [22] reported that metformin acts by directly inhibiting complex I-mediated mitochondrial respiration and citric acid cycle functions in breast cancer cells and their isolated mitochondria. These effects induce a shift in favor of uncoupling reactions, causing mitochondrial metabolism to become energetically inefficient. Thus, metformin inhibits oxygen consumption in isolated mitochondria only in the presence of complex I substrates (malate and pyruvate) but not in the presence of complex II substrates, such as succinate [22]. These data agree with classical evidence showing an inhibitory effect of complex I together with a membrane potential-driven accumulation of positively charged drug within the mitochondrial matrix in hepatocellular carcinoma [23]. Further evidence about the role of complex I in the effects of metformin was provided by Wheaton et al. [24]. These authors showed that metformin exerted an antiproliferative effect in colon cancer cells but that this effect is abolished when a metformin-resistant NADH reductase from *Saccharomyces cerevisiae* (NDI1) was overexpressed. These results were confirmed in mice overexpressing the NDI1 protein [24].

In addition to contributing to an energetic imbalance, the inhibition of mitochondrial complex I has been associated with a decrease in insulin/insulin-like growth factor-1 (IGF-1) signaling, inhibition of mammalian target of rapamycin (mTOR), activation of AMPK (Fig. 1), and reduction in reactive oxygen species (ROS) production and its associated DNA damage [25–28]. AMPK and mTOR signaling comprise a central pathway in tumor proliferation [29] and has been related to metformin activity in such cancers as breast carcinoma [30,31], esophageal cancer [32], pancreatic cancer [33] and gastric cancer [34]. Although the regulatory function of AMPK over mTOR is well established, there is also evidence that metformin can inhibit the activity of mTOR in an AMPK-independent manner, inducing cell cycle arrest through an increase in the expression of the Redd1 protein (Fig. 1) [35]. Nevertheless, AMPK-dependent expression of Redd1 induced by metformin has also been described [36]. Finally, other less well-known effects derived from mTOR inhibition have been suggested to be effects of metformin. Interestingly, recent studies show that metformin has the potential to antagonize the epithelial-mesenchymal transition (EMT) and stemness in cancer cells, an effect that is not an easily predictable consequence of mTOR inhibition [37–39].

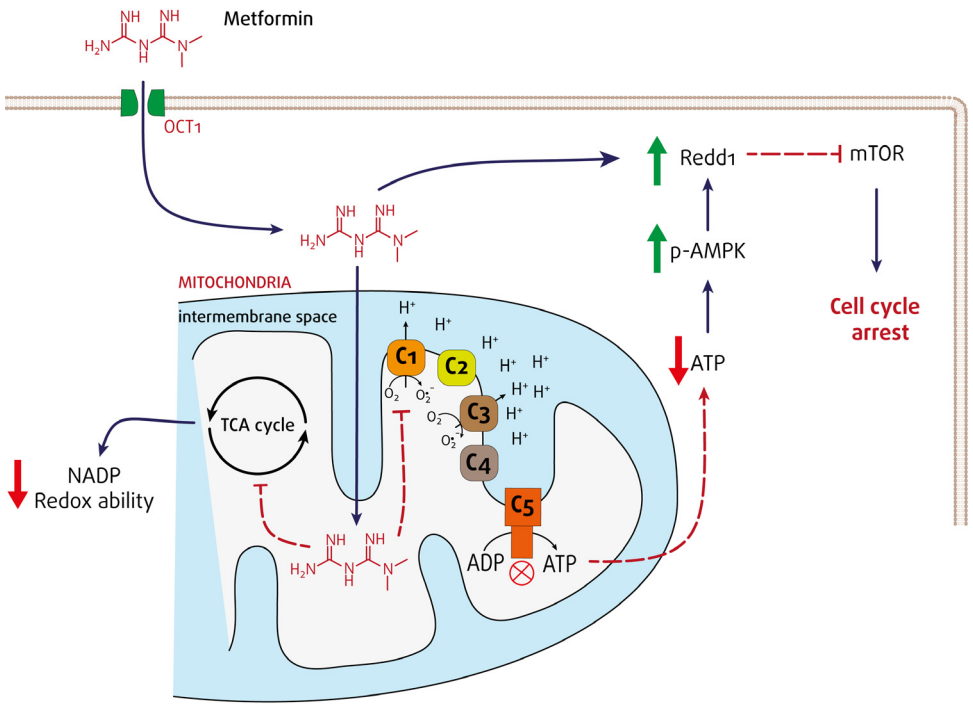

Fig. 1. Proposed metformin's mitochondrial mode of action.
Metformin gets into cancer cells by the Organic cation transporter 1 (OTC1) protein [43]. Discontinuous red lines represent inhibitory or decreasing effect. Blue lines represent activation of signaling pathways. The figure shows the inhibitory effect of metformin on complex I [22], and the next diminution of ATP synthesis in the ATP synthase. Furthermore, metformin induces the activation of AMPK, *via* its phosphorylation, with the consequent inhibition of mTOR and the cell cycle arrest [25,26]. Also has been described that metformin induces Redd1, independently of the activity of AMPK [35]. In addition, metformin has an inhibitory effect on TCA cycle with the subsequent diminution of NADH levels and the diminution of cancer cell redox ability [44].

In addition, metformin-induced bioenergetics alterations in hypoxic tumor cells have been reported. It is important to note that a main and well-known feature of solid tumors is a reduction in vascularization, which affects the availability of both glucose and oxygen and increases the malignant phenotype of tumor cells. Molecularly, the stabilization of hypoxia-inducible factors (HIFs) is a key step in the cascade of metabolic changes that allows adaptation to a low-oxygen environment. Consequently, activation of HIFs (such as HIF-1a) is reported in several types of cancer [40–42]. In this setting, metformin has recently been shown to reduce hypoxia-induced HIF-1a stabilization and the expression of HIF target genes in tumor cells [24]. These effects suggest that metformin may also have a therapeutic role in cancers that is dependent on HIF signaling for survival under hypoxic conditions.

4. Metformin as an "antimetabolite drug"

Folate metabolism has been highlighted as an important target for chemotherapeutic drugs. "Antimetabolite drugs", such as methotrexate and pemetrexed, interfere with folate metabolism, exerting antiproliferative and cytotoxic effects in several types of tumor cells, and are currently used as therapy against several common cancers including breast cancer [45], lung cancer [46] and pediatric leukemia [47]. The importance of folate metabolism in cancer cells involves its relationship with important processes for tumor cell survival and/or chemotherapy resistance in "one-carbon" metabolism, which integrates nucleotide synthesis, the methionine cycle, glutathione synthesis and polyamine metabolism, among other metabolic pathways [48].

The relationship between metformin and folate metabolism alterations is based on several studies reporting an increase in homocysteine and decrease in folate and B12 vitamin in patients with type II diabetes mellitus treated long-term with metformin [49–53]. Homocysteine is linked to folate metabolism by the action of methionine synthase, an enzyme that methylates homocysteine using 5-methyl-tetrahidrofolate as a methyl group donor and B12 vitamin as the main cofactor [48]. However, the relationship between folate metabolism and the antitumor effect of metformin has not been well explored. In this regard, unbiased analyses by HPLC/MS in cancer cells treated with metformin have provided important evidence indicating that the effect of metformin resembles that exerted by antimetabolite drugs, which could explain its antiproliferative and cytotoxic effects (Fig. 2). In 2012, Corominas-Faja et al. reported that metformin alters folate metabolism in breast cancer cells (MCF-7, BT-474 and MDA-MB-231 cell lines), inducing an inhibition of the *de novo* synthesis of thymidine and purine nucleotides as a final cytotoxic effect [54]. This folate metabolism alteration was also linked to a reduction in glutathione synthesis. Metformin in cancer cells also induced an accumulation of homocysteine but decreased the levels of reduced glutathione and the disulfide form of this thiol [54]. These results were further confirmed by Janzer et al. [44], whereby breast cancer stem cells (CSCs) treated with metformin showed an increase in homocysteine levels and a significant decrease in triphosphate nucleotides, as measured by HPLC/MS. Interestingly, in CSCs, metformin does not alter glycolytic intermediates or tricarboxylic acid cycle products, indicating no alteration in mitochondria homeostasis in these cells [44]. In addition, Cioce et al. evaluated the metabolite profile of the culture medium of chemotherapy-resistant breast cancer cells (derived from MCF-7, BT-474 and SUM 159 cell lines) and found that the culture medium from cells treated with metformin had greater levels of pyroglutamate, which agrees with the reduced intracellular levels of glutathione [55]. However, as none of these reports can elucidate the precise mode of action that explains this alteration, specific enzyme inhibition mediated by metformin has not yet been proven.

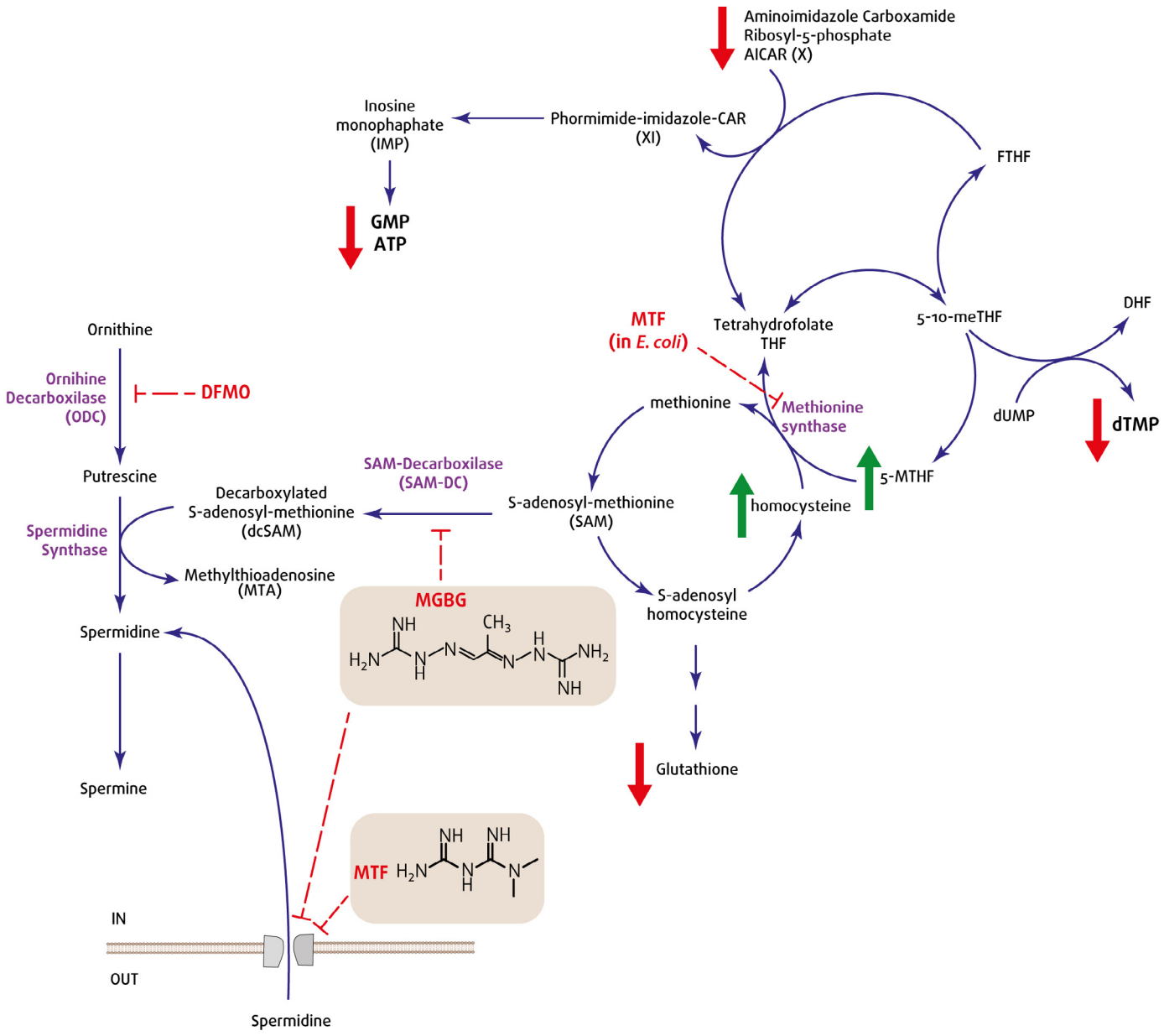

Fig. 2. Effects of metformin in the folate cycle, and its relationship with the methionine cycle and the polyamine metabolism.
Red and green arrows show metabolites that are decreased or accumulated, respectively, by the action of metformin in cancer cells. Discontinuous red lines indicate inhibitory effects. Metformin induces the accumulation of homocysteine and 5-Methyltetrahydrofolate (5-MTHF) [44], whereas decreased the levels of AICAR, glutathione and purine and pyrimidine nucleotides [44,54]. Methylglyoxal bis(guanylhidrazone) (MGBG) and metformin (MTF) inhibit the uptake of spermidine [57]. In the bacteria *E. coli*, metformin (MTF) inhibits the methionine synthase enzyme [56]. DHF: Dihydrofolate. 5-10-meTHF: 5-10-methylene-Tetrahydrofolate. FTHF: 10-Formyl-Tetrahydrofolate. 5-MTHF: 5-Methyltetrahidrofolte. dMTP: Thymidine monophosphate. dUTP: Deoxyuridine monophosphate. GMP: Guanosine monophosphate. ATP: Adenosine triphosphate.

One interesting hypothesis regarding altered folate metabolism is based on other species, namely, experiments performed in a model involving *Caenorhabditis elegans* co-cultured with *Escherichia coli*. In this model, the exposure of *E. coli* to metformin altered folate metabolism, increasing S-adenosylmethionine and 5-methilene-thetrahydrofolate levels, whereas the levels of methionine decreased. Such alterations strongly suggest an inhibition of methionine synthase, leading to the accumulation of homocysteine and 5-MTHF as well as a bacteriostatic effect. In addition, these authors found that this antiproliferative effect in bacteria is not related to energetic metabolism because metformin did not impair respiration in wild-type and ubiquinone-deficient bacteria. The report of Cabreiro et al. is also interesting because it suggests metabolic cross-talk in folate metabolism between the species: an alteration in the folate status of *C. elegans* is produced by the folate alterations in *E. coli*, which is the main source of folate for the nematode. The extrapolation of this information to the human-gut flora interaction could offer interesting approaches to elucidating the effect of metformin on our resident gastrointestinal bacteria [56].

Some evidence also suggests that metformin could be related to polyamine metabolism in mammals. For instance, metformin competitively inhibits the transport of spermidine in NIH 3T3 cells [57]. Thus, because polyamine synthesis is tightly linked to the folate cycle *via* the methionine cycle, this polyamine metabolism impairment could explain the "antimetabolite effect" of metformin. Of note, HPLC/MS metabolomics profiling revealed that difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase (ODC), the first rate-limiting step in polyamine synthesis (Fig. 2), exerts an antiproliferative effect in cancer cells through the accumulation of 5-methyl-THF and reduction of the

thymidine pool. Indeed, thymidine supplementation reverts the cytostatic effects of DFMO in colon cancer cells [58]. How does metformin interfere with polyamine metabolism? Although there is no known interaction between metformin and any enzyme of the polyamine synthesis pathway, the biguanide-class structure of metformin closely resembles methylglyoxal bis(guanylhydrazone) (MGBG), a guanidino-containing drug known to be an inhibitor of S-adenosylmethionine decarboxylase (SAM-DC), exerting antiproliferative and cytotoxic effects in tumor cells [59] (Fig. 2). Similar to metformin, it has been reported that MGBG induces mitochondrial damage independently of SAM-DC inhibition activity [60,61] and lowers glucose levels in humans [62]. Therefore, this sharing of pharmacological effects and the structural similarity between MGBG and metformin suggest that metformin could possess the inhibitory activity of SAM-DC, blocking spermidine synthesis.

Regardless, the described alteration in folate metabolism is not completely counter to the mode of action regarding mitochondria and the AKT pathway. For instance, the report of Janzer et al. shows that treatment with metformin also decreases the levels of all intermediates of the tricarboxylic acid cycle, suggesting that, at least, both phenomena occur at the same time [44]. In addition, Corominas-Faja et al. showed that AMPK activation is a consequence of folate metabolism alteration and thymidine pool depletion, as thymidine or hypoxanthine supplementation restores nucleotide biosynthesis and inhibits the phosphorylation of AMPK, restoring the proliferative ability of cancer cells [54]. Such effects on the AMPK phosphorylation status are also exhibited by methotrexate, pemetrexed and other investigational antifolate drugs that induce apoptosis *via* this kinase [63,64].

5. Other mechanism described for metformin

An additional mode of action for metformin was described with regard to the growth of hepatocellular carcinoma cells (HCCs). In this cancer type, metformin exerts an inhibitory effect both *in vitro* and *in vivo* through the modulation of cell-cycle regulatory proteins, such as cycling D1 and cyclin E [65,66]. Additionally, in HCCs, in which the *C-MYC* gene is suggested to be a pivotal mediator of carcinogenesis [67], metformin treatment has been shown to inhibit *C-MYC* expression *in vitro* by up-regulating tumor suppressor let-7 family microRNAs [66]. Let-7 and lin28 belong to a recently discovered family of microRNAs that are involved the regulation of life span, cell proliferation, differentiation and apoptosis [68]. Furthermore, metformin has also been described as antagonizing lin28 activity, enhancing let-7 levels and antagonizing the carcinogenesis promoted by stem cells [69–71].

Due to the complexity and heterogeneous features of solid tumors, new culture methods, such as mammosphere formation, have offered new tools for studying the effect of drugs in more complex models [72]. Thus, employing cell lines derived from various human breast cancers, metformin at clinically relevant concentrations inhibits mammosphere formation and notably reduces the number of stem cells *in vitro* and also potentiates the cytotoxic activity of doxorubicin in both stem and non-stem-cells [73].

6. *In vitro* versus *in vivo* effects of metformin: the problem of concentration

The most common criticism concerning *in vitro* experiments aimed at explaining the mode of action of metformin is the concentration used. Pharmacokinetic analyses of metformin plasma concentration have revealed that after a regular administration of 3 g/day (maximum dose allowed in therapeutics), the average steady-state concentration is almost 1.5 mg/L; considering the molecular weight of metformin (129.2 g/mol), the average molar concentration in plasma is approximately 11 μM [74,75]. However, experiments probing the mitochondrial activity of metformin have utilized concentrations from 0.5 to 10 mM, at least 50 times greater than the plasma concentrations in humans [22,24,25,76–78]. In addition, some reports provide evidence that the antifolate activity of the drug is observed at concentrations between 0.3 and 1 mM, also greater than the plasma concentration [44,54,55]. Finally, *in vivo* research in which mice are treated with metformin has resulted in interesting evidence. For instance, in mice treated with 250 mg/kg of metformin, resulting in a plasma concentration of 4 mg/mL, Memmot et al. showed that the mTOR pathway was effectively inhibited, whereas phosphor-AMPK was increased, lending strong support to the AMPK-mediated mechanism of action of metformin [27].

Another concern resulting from *in vitro* and *in vivo* data is that glucose in the culture medium could interfere with metformin metabolism. Most of the papers reviewed utilized DMEM or DMEM/F12 as the culture medium, which contain 24 and 17.5 mM glucose, respectively; however, the normal range of glucose concentration in plasma is below 7 mM. In this regard, Zhuang et al. proved that the cytotoxic effect of metformin is potent in low-glucose media, suggesting that the necessity of a high concentration of metformin in *in vitro* experiments is due to the masking effect that glucose exerts; thus, the data obtained in these experiments are not invalidated [76]. This relationship between the effects of metformin and the medium glucose concentration was further confirmed by Weathon et al. [24] and Menendez et al. [78]. Finally, it is obvious that complex metabolism-modifying drugs such as metformin most likely have several modes of action. Thus, a deeper knowledge about these mechanisms could improve the design of new drugs and the understanding of the potential use of metformin in cancer and other multifactorial diseases.

Conflict of interest

The authors declare that there is no conflict of interest associated to this publication.

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References

[1] M.B. Schneider, H. Matsuzaki, J. Haorah, A. Ulrich, J. Standop, X.Z. Ding, T.E. Adrian, P.M. Pour, Prevention of pancreatic cancer induction in hamsters by metformin, Gastroenterology 120 (2001) 1263–1270.
[2] J.M. Evans, L.A. Donnelly, A.M. Emslie-Smith, D.R. Alessi, A.D. Morris, Metformin and reduced risk of cancer in diabetic patients, Br. Med. J. 330 (2005) 1304–1305.
[3] A. Klip, L.A. Leiter, Cellular mechanism of action of metformin, Diabetes Care 13 (1990) 696–704.
[4] S. Song, S. Andrikopoulos, C. Filippis, A.W. Thorburn, D. Khan, J. Proietto, Mechanism of fat-induced hepatic gluconeogenesis: effect of metformin, Am. J. Physiol. Endocrinol. Metab. 281 (2001) E275–E282.
[5] S.A. Hawley, F.A. Ross, C. Chevtzoff, K.A. Green, A. Evans, S. Fogarty, M.C. Towler, L.J. Brown, O.A. Ogunbayo, A.M. Evans, D.G. Hardie, Use of cells expressing gamma subunit variants to identify diverse mechanisms of ampk activation, Cell Metab. 11 (2010) 554–565.
[6] D. Argaud, H. Roth, N. Wiernsperger, X.M. Leverve, Metformin decreases gluconeogenesis by enhancing the pyruvate kinase flux in isolated rat hepatocytes, Eur. J. Biochem. 213 (1993) 1341–1348.
[7] Z. Drahota, E. Palenickova, R. Endlicher, M. Milerova, J. Brejchova, M. Vosahlikova, P. Svoboda, L. Kazdova, M. Kalous, Z. Cervinkova, M. Cahova, Biguanides inhibit complex i, ii and iv of rat liver mitochondria and modify their functional properties, Physiol. Res. 63 (2014) 1–11.

[8] J. Ouyang, R.A. Parakhia, R.S. Ochs, Metformin activates amp kinase through inhibition of amp deaminase, J. Biol. Chem. 286 (2011) 1–11.
[9] G. Zhou, R. Myers, Y. Li, Y. Chen, X. Shen, J. Fenyk-Melody, M. Wu, J. Ventre, T. Doebber, N. Fujii, N. Musi, M.F. Hirshman, L.J. Goodyear, D.E. Moller, Role of amp-activated protein kinase in mechanism of metformin action, J. Clin. Investig. 108 (2001) 1167–1174.
[10] D.G. Hardie, D.R. Alessi, Lkb1 and ampk and the cancer-metabolism link – ten years after, BMC Biol. 11 (2013) 36.
[11] R.A. Miller, Q. Chu, J. Xie, M. Foretz, B. Viollet, M.J. Birnbaum, Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic amp, Nature 494 (2013) 256–260.
[12] J. Cao, S. Meng, E. Chang, K. Beckwith-Fickas, L. Xiong, R.N. Cole, S. Radovick, F.E. Wondisford, L. He, Low concentrations of metformin suppress glucose production in hepatocytes through amp-activated protein kinase (ampk), J. Biol. Chem. 289 (2014) 20435–20446.
[13] A.K. Madiraju, D.M. Erion, Y. Rahimi, X.M. Zhang, D.T. Braddock, R.A. Albright, B.J. Prigaro, J.L. Wood, S. Bhanot, M.J. MacDonald, M.J. Jurczak, J.P. Camporez, H.Y. Lee, G.W. Cline, V.T. Samuel, R.G. Kibbey, G.I. Shulman, Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase, Nature 510 (2014) 542–546.
[14] B.A. Kaipparettu, Y. Ma, J.H. Park, T.L. Lee, Y. Zhang, P. Yotnda, C.J. Creighton, W.Y. Chan, L.J. Wong, Crosstalk from non-cancerous mitochondria can inhibit tumor properties of metastatic cells by suppressing oncogenic pathways, PloS One 8 (2013) e61747.
[15] J.A. Jara, V. Castro, J. Saavedra-Olavarria, L. Peredo, M. Pavani, F. Jana, M.E. Letelier, M.I. Becker, E. Parra, A. Morello, J.D. Maya, J. Ferreira, The antiproliferative and uncoupling effects of delocalized, lipophilic, cationic gallic acid derivatives on cancer cell lines. Validation in vivo in singenic mice, J. Med. Chem. 57 (2014) 2440–2454.
[16] H. Yuhara, C. Steinmaus, S.E. Cohen, D.A. Corley, Y. Tei, P.A. Buffler, Is diabetes mellitus an independent risk factor for colon cancer and rectal cancer? Am. J. Gastroenterol. 106 (2011) 1911–1921, quiz 1922.
[17] H.U. Krämer, B. Schöttker, E. Raum, H. Brenner, Type 2 diabetes mellitus and colorectal cancer: meta-analysis on sex-specific differences, Eur. J. Cancer 48 (2012) 1269–1282.
[18] R. Vatrinet, L. Iommarini, I. Kurelac, M. De Luise, G. Gasparre, A.M. Porcelli, Targeting respiratory complex i to prevent the warburg effect, Int. J. Biochem. Cell Biol. 63 (2015) 41–45.
[19] A. Isakovic, L. Harhaji, D. Stevanovic, Z. Markovic, M. Sumarac-Dumanovic, V. Starcevic, D. Micic, V. Trajkovic, Dual antiglioma action of metformin: cell cycle arrest and mitochondria-dependent apoptosis, Cell. Mol. Life Sci. 64 (2007) 1290–1302.
[20] C.C. Beeson, G.C. Beeson, R.G. Schnellmann, A high-throughput respirometric assay for mitochondrial biogenesis and toxicity, Anal. Biochem. 404 (2010) 75–81.
[21] R. Sanchez-Alvarez, U.E. Martinez-Outschoorn, R. Lamb, J. Hulit, A. Howell, R. Gandara, M. Sartini, E. Rubin, M.P. Lisanti, F. Sotgia, Mitochondrial dysfunction in breast cancer cells prevents tumor growth: understanding chemoprevention with metformin, Cell Cycle 12 (2013) 172–182.
[22] S. Andrzejewski, S.P. Gravel, M. Pollak, J. St-Pierre, Metformin directly acts on mitochondria to alter cellular bioenergetics, Cancer Metab. 2 (2014) 12.
[23] M.R. Owen, E. Doran, A.P. Halestrap, Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain, Biochem. J. 348 (Pt 3) (2000) 607–614.
[24] W.W. Wheaton, S.E. Weinberg, R.B. Hamanaka, S. Soberanes, L.B. Sullivan, E. Anso, A. Glasauer, E. Dufour, G.M. Mutlu, G.S. Budigner, N.S. Chandel, Metformin inhibits mitochondrial complex i of cancer cells to reduce tumorigenesis, eLife 3 (2014) e20224.
[25] C. Algire, O. Moiseeva, X. Deschênes-Simard, L. Amrein, L. Petruccelli, E. Birman, B. Viollet, G. Ferbeyre, M.N. Pollak, Metformin reduces endogenous reactive oxygen species and associated DNA damage, Cancer Prev. Res. 5 (2012) 536–543.
[26] D.M. Gwinn, D.B. Shackelford, D.F. Egan, M.M. Mihaylova, A. Mery, D.S. Vasquez, B.E. Turk, R.J. Shaw, Ampk phosphorylation of raptor mediates a metabolic checkpoint, Mol. Cell 30 (2008) 214–226.
[27] R.M. Memmott, J.R. Mercado, C.R. Maier, S. Kawabata, S.D. Fox, P.A. Dennis, Metformin prevents tobacco carcinogen-induced lung tumorigenesis, Cancer Prev. Res. 3 (2010) 1066–1076.
[28] S. Gandini, M. Puntoni, B.M. Heckman-Stoddard, B.K. Dunn, L. Ford, A. DeCensi, E. Szabo, Metformin and cancer risk and mortality: a systematic review and meta-analysis taking into account biases and confounders, Cancer Prev. Res. 7 (2014) 867–885.
[29] B. Faubert, E.E. Vincent, M.C. Poffenberger, R.G. Jones, The amp-activated protein kinase (ampk) and cancer: many faces of a metabolic regulator, Cancer Lett. 356 (2015) 165–170.
[30] L.S. Guo, H.X. Li, C.Y. Li, S.Y. Zhang, J. Chen, Q.L. Wang, J.M. Gao, J.Q. Liang, M.T. Gao, Y.J. Wu, Synergistic antitumor activity of vitamin d3 combined with metformin in human breast carcinoma mda-mb-231 cells involves m-tor related signaling pathways, Pharmazie 70 (2015) 117–122.
[31] R.J. Dowling, M. Zakikhani, I.G. Fantus, M. Pollak, N. Sonenberg, Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells, Cancer Res. 67 (2007) 10804–10812.
[32] S. Honjo, J.A. Ajani, A.W. Scott, Q. Chen, H.D. Skinner, J. Stroehlein, R.L. Johnson, S. Song, Metformin sensitizes chemotherapy by targeting cancer stem cells and the mtor pathway in esophageal cancer, Int. J. Oncol. 45 (2014) 567–574.
[33] V. Nair, S. Sreevalsan, R. Basha, M. Abdelrahim, A. Abudayyeh, A. Rodrigues Hoffman, S. Safe, Mechanism of metformin-dependent inhibition of mammalian target of rapamycin (mtor) and ras activity in pancreatic cancer: role of specificity protein (sp) transcription factors, J. Biol. Chem. 289 (2014) 27692–27701.
[34] G. Han, H. Gong, Y. Wang, S. Guo, K. Liu, Ampk/mtor-mediated inhibition of survivin partly contributes to metformin-induced apoptosis in human gastric cancer cell, Cancer Biol. Ther. 16 (2015) 77–87.
[35] I. Ben Sahra, C. Regazzetti, G. Robert, K. Laurent, Y. Le Marchand-Brustel, P. Auberger, J.F. Tanti, S. Giorgetti-Peraldi, F. Bost, Metformin, independent of ampk, induces mtor inhibition and cell-cycle arrest through redd1, Cancer Res. 71 (2011) 4366–4372.
[36] J. Sesen, P. Dahan, S.J. Scotland, E. Saland, V.T. Dang, A. Lemarie, B.M. Tyler, H. Brem, C. Toulas, E. Cohen-Jonathan Moyal, J.E. Sarry, N. Skuli, Metformin inhibits growth of human glioblastoma cells and enhances therapeutic response, PloS One 10 (2015) e0123721.
[37] A. Vazquez-Martin, C. Oliveras-Ferraros, S. Cufí, S. Del Barco, B. Martin-Castillo, J.A. Menendez, Metformin regulates breast cancer stem cell ontogeny by transcriptional regulation of the epithelial-mesenchymal transition (emt) status, Cell Cycle 9 (2010) 3807–3814.
[38] S. Cufí, A. Vazquez-Martin, C. Oliveras-Ferraros, B. Martin-Castillo, J. Joven, J.A. Menendez, Metformin against tgfβ-induced epithelial-to-mesenchymal transition (emt): from cancer stem cells to aging-associated fibrosis, Cell Cycle 9 (2010) 4461–4468.
[39] A. Vazquez-Martin, C. Oliveras-Ferraros, S. Del Barco, B. Martin-Castillo, J.A. Menendez, The anti-diabetic drug metformin suppresses self-renewal and proliferation of trastuzumab-resistant tumor-initiating breast cancer stem cells, Breast Cancer Res. Treat. 126 (2011) 355–364.
[40] S. Yoshiba, D. Ito, T. Nagumo, T. Shirota, M. Hatori, S. Shintani, Hypoxia induces resistance to 5-fluorouracil in oral cancer cells via g(1) phase cell cycle arrest, Oral Oncol. 45 (2009) 109–115.
[41] J. Xiong, F.F. Zhu, M.F. Nie, Hypoxia-inducible factor-2α (hif-2α) mediates the effects of hypoxia on the promotion of hela cell viability, colony formation, and invasion capacity in vitro, Genet. Mol. Res. 14 (2015) 3281–3292.
[42] M. Maftouh, A. Avan, R. Sciarrillo, C. Granchi, L.G. Leon, R. Rani, N. Funel, K. Smid, R. Honeywell, U. Boggi, F. Minutolo, G.J. Peters, E. Giovannetti, Synergistic interaction of novel lactate dehydrogenase inhibitors with gemcitabine against pancreatic cancer cells in hypoxia, Br. J. Cancer 110 (2014) 172–182.
[43] Y. Shu, S.A. Sheardown, C. Brown, R.P. Owen, S. Zhang, R.A. Castro, A.G. Ianculescu, L. Yue, J.C. Lo, E.G. Burchard, C.M. Brett, K.M. Giacomini, Effect of genetic variation in the organic cation transporter 1 (oct1) on metformin action, J. Clin. Investig. 117 (2007) 1422–1431.
[44] A. Janzer, N.J. German, K.N. Gonzalez-Herrera, J.M. Asara, M.C. Haigis, K. Struhl, Metformin and phenformin deplete tricarboxylic acid cycle and glycolytic intermediates during cell transformation and ntps in cancer stem cells, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) 10574–10579.
[45] S. Gandhi, G.G. Fletcher, A. Eisen, M. Mates, O.C. Freedman, S.F. Dent, M.E. Trudeau, Adjuvant chemotherapy for early female breast cancer: a systematic review of the evidence for the 2014 cancer care ontario systemic therapy guideline, Curr. Oncol. 22 (2015) S82–S94.
[46] G. Pilkington, A. Boland, T. Brown, J. Oyee, A. Bagust, R. Dickson, A systematic review of the clinical effectiveness of first-line chemotherapy for adult patients with locally advanced or metastatic non-small cell lung cancer, Thorax 70 (2015) 359–367.
[47] K. Schmiegelow, S.N. Nielsen, T.L. Frandsen, J. Nersting, Mercaptopurine/methotrexate maintenance therapy of childhood acute lymphoblastic leukemia: clinical facts and fiction, J. Pediatr. Hematol./Oncol. 36 (2014) 503–517.
[48] J.W. Locasale, Serine glycine and one-carbon units: cancer metabolism in full circle, Nat. Rev. Cancer 13 (2013) 572–583.
[49] A.K. Aarsand, S.M. Carlsen, Folate administration reduces circulating homocysteine levels in niddm patients on long-term metformin treatment, J. Intern. Med. 244 (1998) 169–174.
[50] M. Sahin, N.B. Tutuncu, D. Ertugrul, N. Tanaci, N.D. Guvener, Effects of metformin or rosiglitazone on serum concentrations of homocysteine, folate, and vitamin b12 in patients with type 2 diabetes mellitus, J. Diabetes Complications 21 (2007) 118–123.
[51] J. De Jager, A. Kooy, P. Lehert, M.G. Wulffele, J. van der Kolk, D. Bets, J. Verburg, A.J. Donker, C.D. Stehouwer, Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin b-12 deficiency: randomised placebo controlled trial, Br. Med. J. 340 (2010) c2181.
[52] S.H. Ko, S.H. Ko, Y.B. Ahn, K.H. Song, K.D. Han, Y.M. Park, S.H. Ko, H.S. Kim, Association of vitamin b12 deficiency and metformin use in patients with type 2 diabetes, J. Korean Med. Sci. 29 (2014) 965–972.
[53] A.C. Ham, A.W. Enneman, S.C. van Dijk, S. Oliai Araghi, K.M. Swart, E. Sohl, J.P. van Wijngaarden, N.L. van der Zwaluw, E.M. Brouwer-Brolsma, R.A. Dhonukshe-Rutten, N.M. van Schoor, T.J. van der Cammen, M.C. Zillikens, R. de Jonge, P. Lips, L.C. de Groot, J.B. van Meurs, A.G. Uitterlinden, R.F. Witkamp, B.H. Stricker, N. van der Velde, Associations between medication use and homocysteine levels in an older population, and potential mediation by vitamin b12 and folate: data from the b-proof study, Drugs Aging 31 (2014) 611–621.
[54] B. Corominas-Faja, R. Quirantes-Pine, C. Oliveras-Ferraros, A. Vazquez-Martin, S. Cufi, B. Martin-Castillo, V. Micol, J. Joven, A. Segura-Carretero, J.A. Menendez, Metabolomic fingerprint reveals that metformin impairs

one-carbon metabolism in a manner similar to the antifolate class of chemotherapy drugs, Aging 4 (2012) 480–498.
[55] M. Cioce, M. Valerio, L. Casadei, C. Pulito, A. Sacconi, F. Mori, F. Biagioni, C. Manetti, P. Muti, S. Strano, G. Blandino, Metformin-induced metabolic reprogramming of chemoresistant aldhbright breast cancer cells, Oncotarget 5 (2014) 4129–4143.
[56] F. Cabreiro, C. Au, K.Y. Leung, N. Vergara-Irigaray, H.M. Cocheme, T. Noori, D. Weinkove, E. Schuster, N.D. Greene, D. Gems, Metformin retards aging in *C. elegans* by altering microbial folate and methionine metabolism, Cell 153 (2013) 228–239.
[57] N.A. Khan, N. Wiernsperger, V. Quemener, R. Havouis, J.P. Moulinoux, Characterization of metformin transport system in nih 3t3 cells, J. Cell. Physiol. 152 (1992) 310–316.
[58] M. Witherspoon, Q. Chen, L. Kopelovich, S.S. Gross, S.M. Lipkin, Unbiased metabolite profiling indicates that a diminished thymidine pool is the underlying mechanism of colon cancer chemoprevention by alpha-difluoromethylornithine, Cancer Discov. 3 (2013) 1072–1081.
[59] S. Ekelund, P. Nygren, R. Larsson, Guanidino-containing drugs in cancer chemotherapy: biochemical and clinical pharmacology, Biochem. Pharmacol. 61 (2001) 1183–1193.
[60] A. Pleshkewych, D.L. Kramer, E. Kelly, C.W. Porter, Independence of drug action on mitochondria and polyamines in l1210 leukemia cells treated with methylglyoxal-bis(guanylhydrazone), Cancer Res. 40 (1980) 4533–4540.
[61] C. Loesberg, H. Van Rooji, J.C. Romijn, L.A. Smets, Mitochondrial effects of the guanidino group-containing cytostatic drugs, m-iodobenzylguanidine and methylglyoxal bis (guanylhydrazone), Biochem. Pharmacol. 42 (1991) 793–798.
[62] E. Mihich, W. Regelson, L.S. Englander, G. Costa, O. Selawry, J.F. Holland, Hypoglycemic effects of methylglyoxal bis (guanylhydrazone) in animals and man, Cancer Chemother. Rep. Part 1 16 (1962) 177–181.
[63] S.B. Rothbart, A.C. Racanelli, R.G. Moran, Pemetrexed indirectly activates the metabolic kinase ampk in human carcinomas, Cancer Res. 70 (2010) 10299–10309.
[64] A.C. Racanelli, S.B. Rothbart, C.L. Heyer, R.G. Moran, Therapeutics by cytotoxic metabolite accumulation: pemetrexed causes zmp accumulation, ampk activation, and mammalian target of rapamycin inhibition, Cancer Res. 69 (2009) 5467–5474.
[65] Z. Qu, Y. Zhang, M. Liao, Y. Chen, J. Zhao, Y. Pan, In vitro and in vivo antitumoral action of metformin on hepatocellular carcinoma, Hepatol. Res. 42 (2012) 922–933.
[66] H. Miyoshi, K. Kato, H. Iwama, E. Maeda, T. Sakamoto, K. Fujita, Y. Toyota, J. Tani, T. Nomura, S. Mimura, M. Kobayashi, A. Morishita, H. Kobara, H. Mori, H. Yoneyama, A. Deguchi, T. Himoto, K. Kurokohchi, K. Okano, Y. Suzuki, K. Murao, T. Masaki, Effect of the anti-diabetic drug metformin in hepatocellular carcinoma in vitro and in vivo, Int. J. Oncol. 45 (2014) 322–332.
[67] G. Blandino, M. Valerio, M. Cioce, F. Mori, L. Casadei, C. Pulito, A. Sacconi, F. Biagioni, G. Cortese, S. Galanti, C. Manetti, G. Citro, P. Muti, S. Strano, Metformin elicits anticancer effects through the sequential modulation of dicer and c-myc, Nat. Commun. 3 (2012) 865.
[68] F. Rehfeld, A.M. Rohde, D.T. Nguyen, F.G. Wulczyn, Lin28 and let-7: ancient milestones on the road from pluripotency to neurogenesis, Cell Tissue Res. 359 (2015) 145–160.
[69] J.A. West, S.R. Viswanathan, A. Yabuuchi, K. Cunniff, A. Takeuchi, I.H. Park, J.E. Sero, H. Zhu, A. Perez-Atayde, A.L. Frazier, M.A. Surani, G.Q. Daley, A role for lin28 in primordial germ-cell development and germ-cell malignancy, Nature 460 (2009) 909–913.
[70] A. Serguienko, I. Grad, A.B. Wennerstrøm, L.A. Meza-Zepeda, B. Thiede, E.W. Stratford, O. Myklebost, E. Munthe, Metabolic reprogramming of metastatic breast cancer and melanoma by let-7a microrna, Oncotarget 6 (2015) 2451–2465.
[71] Q. Hu, J. Peng, W. Liu, X. He, L. Cui, X. Chen, M. Yang, H. Liu, S. Liu, H. Wang, Lin28b is a novel prognostic marker in gastric adenocarcinoma, Int. J. Clin. Exp. Pathol. 7 (2014) 5083–5092.
[72] J. Manuel Iglesias, I. Beloqui, F. Garcia-Garcia, O. Leis, A. Vazquez-Martin, A. Eguiara, S. Cufi, A. Pavon, J.A. Menendez, J. Dopazo, A.G. Martin, Mammosphere formation in breast carcinoma cell lines depends upon expression of e-cadherin, PloS One 8 (2013) e77281.
[73] H.A. Hirsch, D. Iliopoulos, P.N. Tsichlis, K. Struhl, Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission, Cancer Res. 69 (2009) 7507–7511.
[74] G.G. Graham, J. Punt, M. Arora, R.O. Day, M.P. Doogue, J.K. Duong, T.J. Furlong, J.R. Greenfield, L.C. Greenup, C.M. Kirkpatrick, J.E. Ray, P. Timmins, K.M. Williams, Clinical pharmacokinetics of metformin, Clin. Pharmacokint. 50 (2011) 81–98.
[75] J.K. Duong, S.S. Kumar, C.M. Kirkpatrick, L.C. Greenup, M. Arora, T.C. Lee, P. Timmins, G.G. Graham, T.J. Furlong, J.R. Greenfield, K.M. Williams, R.O. Day, Population pharmacokinetics of metformin in healthy subjects and patients with type 2 diabetes mellitus: simulation of doses according to renal function, Clin. Pharmacokinet. 52 (2013) 373–384.
[76] Y. Zhuang, D.K. Chan, A.B. Haugrud, W.K. Miskimins, Mechanisms by which low glucose enhances the cytotoxicity of metformin to cancer cells both in vitro and in vivo, PloS One 9 (2014) e108444.
[77] M. Zakikhani, R. Dowling, I.G. Fantus, N. Sonenberg, M. Pollak, Metformin is an amp kinase-dependent growth inhibitor for breast cancer cells, Cancer Res. 66 (2006) 10269–10273.
[78] J.A. Menendez, C. Oliveras-Ferraros, S. Cufi, B. Corominas-Faja, J. Joven, B. Martin-Castillo, A. Vazquez-Martin, Metformin is synthetically lethal with glucose withdrawal in cancer cells, Cell Cycle 11 (2012) 2782–2792.