

Metformin, cancer and glucose metabolism

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Abstract

Metformin is the first-line treatment for type 2 diabetes. Results from several clinical studies have indicated that type 2 diabetic patients treated with metformin might have a lower cancer risk. One of the primary metabolic changes observed in malignant cell transformation is an increased catabolic glucose metabolism. In this context, once it has entered the cell through organic cation transporters, metformin decreases mitochondrial respiration chain activity and ATP production that, in turn, activates AMP-activated protein kinase, which regulates energy homeostasis. In addition, metformin reduces cellular energy availability and glucose entrapment by inhibiting hexokinase-II, which catalyses the glucose phosphorylation reaction. In this review, we discuss recent findings on molecular mechanisms that sustain the anticancer effect of metformin through regulation of glucose metabolism. In particular, we have focused on the emerging action of metformin on glycolysis in normal and cancer cells, with a drug discovery perspective.

Key Words

- ▶ Metformin
- ▶ Cancer
- ▶ Glucose
- ▶ Hexokinase
- ▶ AMPK

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Introduction

Metformin is a biguanide, whose chemical scaffold was discovered by the extraction of the galegine, a guanidine analogue, from French lilac (*Galega officinalis*) plants. Metformin is the first-line therapy for type 2 diabetes (Alexander *et al.* 2008). In 2005, a 23% reduction in the incidence of any cancer in type 2 diabetic patients treated with metformin was reported (Evans *et al.* 2005). In recent years, results from retrospective epidemiological and *in vitro* studies have supported the rationale of designing clinical trials using metformin as an adjuvant in chemotherapy for cancer patients (Zhang *et al.* 2013). Conversely, the recently raised criticisms about the role

of metformin in cancer have underlined the clinical heterogeneity between trials and the presence of time-related biases (Stevens *et al.* 2012, Suissa & Azoulay 2012, Badrick & Renehan 2014). Indeed, recent experimental results have shed new light on the mechanisms of action of metformin as a key regulator of cellular metabolism. It has been reported that metformin inhibits proliferation and induces apoptosis in cancer cells as a result of decreased energy disposition due to an increased AMP:ATP ratio and AMP-activated protein kinase (AMPK) activation (Viollet *et al.* 2011). The ability of metformin to mimic a condition of caloric restriction is currently of great interest in the

field of oncology (Omar *et al.* 2010). In fact, to compensate the rapid cell growth and proliferation, cancer cells exploit all possible mechanisms, including increased metabolism, demand for nutrients and consumption of glucose, which is known as the Warburg effect (Koppenol *et al.* 2011). Results of recent studies have indicated that metformin treatment might inhibit glucose uptake in tumours. In this review, we describe findings supporting the effect of metformin on glucose metabolism in normal and cancer cells.

Metformin and organic cation transporters in diabetes and cancer

In diabetic patients, metformin decreases plasma glucose concentration mainly by decreasing hepatic gluconeogenesis and glucose output (Wollen & Bailey 1988). This effect is followed by an increase in glucose uptake (Iozzo *et al.* 2003) and the amelioration of insulin resistance. Metformin treatment, through AMPK activation, increases insulin-activated but not basal glucose uptake in muscle (Hundal *et al.* 1992, Sajan *et al.* 2010, Kristensen *et al.* 2014). In all cases, the potentiation of glucose uptake in insulin target tissues was mostly observed after long-term treatment with metformin and the effect was associated with restoration of insulin sensitivity (Natali & Ferrannini 2006). Owing to its physicochemical properties, metformin is not transported across biological membranes by passive diffusion but mainly by organic cation transporters (OCTs), which are therefore determinants of metformin disposition and therapeutic efficacy. The fact that the main responsive tissue to metformin is the liver is supported by the expression pattern of the principal metformin transporter, the solute carrier family 22 member 1 (SLC22A1), also known as OCT1, which is almost exclusively expressed in the liver (Zhang *et al.* 1997). Two other OCT isoforms have been identified. Cellular intake of metformin can be mediated also by OCT2 (gene *SLC22A2*) and OCT3 (gene *SLC22A3*). OCT2 is absent in the liver and highly expressed in renal tubules where it mediates the uptake of metformin from the circulation into renal epithelial cells. Compared with OCT1 and OCT2, OCT3 shows a much broader tissue distribution and it has been proposed to be a new transporter of metformin in skeletal muscle, heart, brain, placenta and liver (Aoki *et al.* 2008, Chen *et al.* 2010). Excluding adrenal gland, OCT3 is weakly expressed in other tissues including liver where the *OCT1:OCT3* mRNA ratio reaches 34 (Nies *et al.* 2009). Hepatic uptake of metformin is primarily mediated by OCT1. Liver accumulates the highest amount of metformin via the portal

circulation and the high level of expression of OCT1. At the same time, OCT2 plays an important role in the disposition of metformin because renal secretion is the major elimination route of metformin. Metformin in fact is not metabolised but excreted unchanged in the urine with a half-life of about 6 h (Graham *et al.* 2011). It is important to note that OCT1 and OCT2 expression levels as well as their genetic polymorphisms contribute to the inter-individual variability in response to metformin treatment (Shu *et al.* 2008, Takane *et al.* 2008). Indeed, it has been shown that several genetic variations in OCT1 and OCT2 are associated respectively with a reduced metformin uptake or increased elimination causing significant changes in plasma concentrations of metformin and hypoglycaemic effects (Choi & Song 2008). Combined impairment of OCT1 and OCT2 in double-knockout mice decreases hepatic metformin uptake to 76% (Higgins *et al.* 2012). Compensatory mechanisms such as passive diffusion seem unlikely because of the polarity and low membrane permeability of metformin. While the virtual absence of OCT1 expression is associated with noncancerous tissues, tumour cells significantly express OCT1 and its downregulation limits the anti-neoplastic effect of metformin (Segal *et al.* 2011, Gupta *et al.* 2012). In this scenario, metformin's ability to increase OCT1 expression may represent an important tool for potential applications in oncology (Salani *et al.* 2012). For instance, pyriplatin, which is a member of the platinum analogues and has never been tested in humans, provides an example of a drug distinct from metformin, for which OCT1 may play a role in its pharmacokinetic and toxicity. Using OCT1 knockout mice, it has been demonstrated that OCT1 expression facilitates the accumulation of pyriplatin (Li *et al.* 2011). Also, tyrosine kinase inhibitors such as imatinib, used in the treatment of chronic myeloid leukemia, are transported by OCT1 (Echoute *et al.* 2011), though the relationship between OCT1 genetic profile and drug responsiveness is still debated (Grinfeld *et al.* 2013, Koren-Michowitz *et al.* 2013, Nies *et al.* 2014). Further studies on genetic variants of OCT1 could help to predict the efficacy of both metformin and platinum-based drugs (Zhang *et al.* 2006).

Metformin and glucose cell metabolism

Canonical effects of metformin on glucose metabolism

One of the most discussed therapeutic actions of metformin in diabetes is the inhibition of gluconeogenesis and hepatic glucose output obtained by the disruption of

energy metabolism, mainly characterised by an increase in the AMP:ATP ratio (El-Mir *et al.* 2000). These results indicate that AMPK, a serine/threonine kinase that regulates cellular homeostasis in response to energy stress and energy unbalance, is the primary target of metformin (Viollet *et al.* 2011). AMPK is a hetero-trimetric protein composed of one catalytic (α) and two regulatory subunits (β and γ). When intracellular ATP concentration decreases, AMP or ADP binds the γ regulatory subunit leading to a conformational change that protect against phosphorylation of AMPK in Thr172 by the serine/threonine kinase LKB1 (Hawley *et al.* 2003) or in response to calcium flux via CAMKK2 kinase (Hawley *et al.* 2005). Metformin treatment and caloric restriction increase the AMP:ATP ratio and activate AMPK, switching cells from an anabolic to a catabolic state. In particular, through AMPK pathway, metformin induces the phosphorylation of CREB-regulated transcription co-activator 2 (CRTC2 or TORC2), sequestering CRTC2 from the nucleus to the cytoplasm (Shaw *et al.* 2005). This mechanism inhibits the transcriptional activation of gluconeogenic genes such as phosphoenolpyruvate (PEP) carboxykinase (PEPCK) (PCK2) and glucose-6-phosphatase (G6Pase). Whether AMPK is absolutely required to decrease circulating plasma glucose concentration remains controversial. Metformin, in fact, can also decrease hepatic gluconeogenesis independently of the LKB1/AMPK pathway as demonstrated in AMPK- and LKB1-deficient hepatocytes (Foretz *et al.* 2010). The inhibition of liver gluconeogenesis in these models seems related to energy state changes associated with mitochondrial function and does not involve gluconeogenic gene expression processes.

The multiplicity of effects of metformin also includes the reduction in gluconeogenesis in liver cells due to the decreased uptake of gluconeogenic substrates such as L-alanine (Komori *et al.* 1993) and lactate (Radziuk *et al.* 1997) and the direct inhibition of the redox shuttle enzyme glycerophosphate dehydrogenase (Madiraju *et al.* 2014). Metformin also reduces glucose output through the decrease in cAMP, protein kinase A activity and phosphorylation of protein kinase A substrates induced by glucagon (Miller *et al.* 2013). The stimulatory effect of metformin on glycogenolysis and glycolysis is the consequence of reduced oxidative phosphorylation coupled with the inhibition of oxygen consumption. These effects do not necessarily require AMPK activation because changes in AMP:ATP ratio can allosterically influence the enzymatic activity of several enzymes of the glycolytic pathway. High levels of ATP, for instance, allosterically

inhibit phosphofructokinase and pyruvate kinase, which are important control elements in glycolysis.

Respiratory chain inhibition has been identified as the first mechanism by which metformin induces significant changes in total cellular ATP content (El-Mir *et al.* 2000). Observing the decreased oxygen consumption and mitochondrial membrane potential in permeabilised cells and isolated mitochondria, the existence of a direct effect on complex 1 of the respiratory chain was proposed (Owen *et al.* 2000). This effect could be the result of molecular changes occurring in the inner mitochondrial membrane due to the binding of metformin to phospholipids (Drahota *et al.* 2014). Modification of membrane permeability and fluidity may induce changes in mitochondrial proteins and functionality by interfering with the V_{\max} of catalysis and the conformations of important mitochondrial proteins, including respiratory chain complex 1. These indirect mechanisms could promote mitochondrial injury and result in a predisposition to cell death.

Effect of metformin on glucose metabolism in cancer

The first identified cellular target of metformin linking AMPK and cancer came from the discovery of *LKB1*, a tumour suppressor gene, which was identified as the major upstream activator of AMPK (Shaw *et al.* 2004). The LKB1–AMPK interaction mediates glucose homeostasis in liver and play an important role in the suppression of protein synthesis, a process often deregulated in cancer (Zakikhani *et al.* 2006). Activation of LKB1–AMPK induced by metformin results in the inhibition of Raptor–mTOR (mammalian TOR complex 1(mTORC1)) complex and suppression of cellular protein synthesis and growth (Dowling *et al.* 2007). Phosphorylation of AMPK activates tuberous sclerosis complex 2 (TSC2), a subunit of the TSC1–TSC2 (hamartin/tuberin) complex. The TSC1–TSC2 complex, through its GTPase-activating protein, inactivates the small GTP-binding protein Ras Homolog Enriched in Brain (RHEB). Once bound to GDP, RHEB cannot activate mTORC1, which decreases mRNA translation and ribosome biogenesis affecting the cell cycle and proliferation (Inoki *et al.* 2006). Metformin can exert its anti-neoplastic effect by inhibiting pathways downstream of AMPK without any activation of AMPK, as demonstrated in lung cancer (Memmott *et al.* 2010). AMPK-independent effects of metformin in cancer, such as the inhibitory effect on AKT phosphorylation in breast and lung cancers, has been well documented and associated also with decreased plasma concentrations of insulin-like

growth factor 1 (IGF1) and insulin (Pollak 2012). Thus, the actions of metformin are not exclusively mediated by inhibition of AMPK (Watson 2014).

Indeed, in recent years new effects of metformin upstream of AMPK activation have been identified. In particular, metabolic changes have been rediscovered in the context of cancer diseases after the initial observations of Otto Warburg, who noticed that proliferating cancer cells consume glucose at a high rate, releasing lactate and not carbon dioxide (Koppenol *et al.* 2011). The tumour cells avidly use glucose as a source of energy and for production of cell-building blocks. Critical to this phenotype is the production of glucose-6-phosphate (G6P), whose role in glucose retention and metabolism is highly advantageous for cancer cell survival and proliferation (Vander Heiden *et al.* 2009). Glucose phosphorylation is the first step in glucose metabolism. This reaction is catalysed by hexokinases (HKs), enzymes that exist in four isozymic forms, differing in catalytic and regulatory properties as well as subcellular localisation. HK1, -2 and 3 are 100 kDa molecules that show a high affinity for glucose, as demonstrated by their K_m values in the micromolar range, and are very sensitive to the inhibition by their product G6P. The type 1 isozyme is ubiquitously expressed, while the type 2 is found in skeletal muscle, adipose tissue and heart; the type 3 is limited to neurons. In contrast, HK4, also referred as glucokinase, is expressed in liver and pancreas. HK4 has a molecular mass of 50 kDa,

exhibits a lower affinity for glucose and represents the only HK that is not inhibited by G6P (Wilson 2003). HKs 1 and 2 have been found in mitochondrial fraction of several tissues (Wilson 2003). Binding of HKs 1 and 2 to mitochondria represents a privileged access to ATP produced by the respiratory chain. In the outer mitochondrial membrane, in fact, HK1 and 2 interact with the permeability transition pore which includes the voltage-dependent anion channel 1 (VDAC1) responsible for ATP flux to the cytoplasm. This physical association facilitates glucose phosphorylation and protects cell from apoptosis (Pastorino & Hoek 2003). HK2 is highly expressed in lung and breast cancer and is required by proliferating cancer cells as demonstrated by the impairment of tumour progression following its downregulation (Fang *et al.* 2012, Patra *et al.* 2013). In the unbound state, HK2 exists in an open inactive structure (Fig. 1a), where ATP is bound within the large lobe but in a conformation far from the catalytic site. Upon glucose binding, a large conformational change closes the two lobes resulting in an enzymatically active closed HK2 that presents ATP in a productive conformation protruding towards the sugar substrate (Fig. 1). Metformin directly inhibits HK2 activity and subcellular localisation inducing dissociation of HK2 from the mitochondria (Fig. 2). The region occupied by metformin has been simulated by computational modelling and found to correspond with the binding site of G6P. Accordingly, it was found that metformin does not inhibit glucokinase

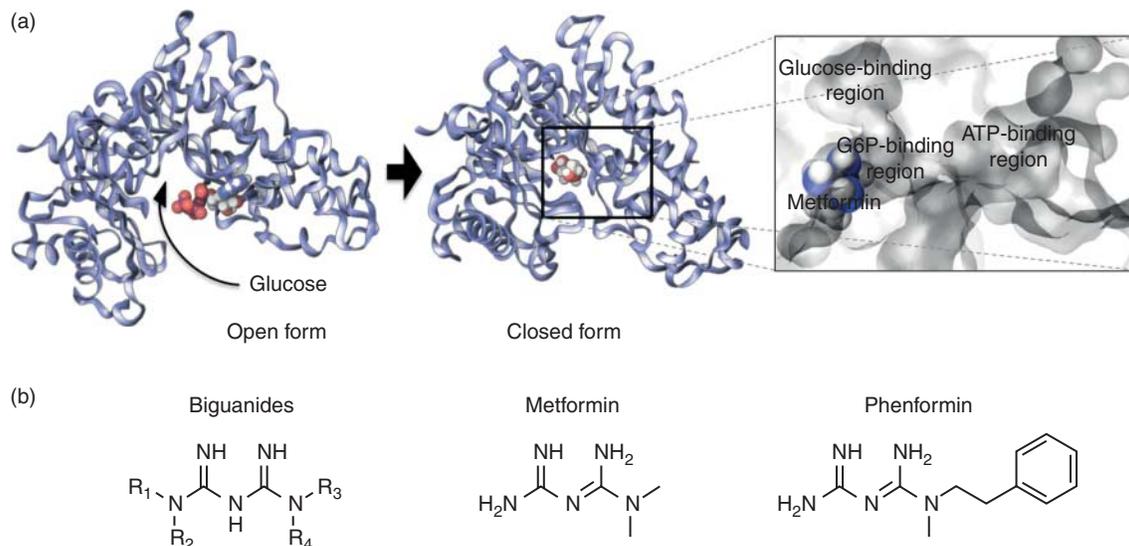


Figure 1

(a) Crystallographic structure of HK2 in open (based on PDB ID: 1IG8) and closed (based on PDB ID: 2NZT) conformations in complex with glucose and ATP and a close-up picture of the catalytic site with the putative binding

mode of metformin in the G6P binding region. (b) Chemical structure of biguanide analogues, metformin and phenformin.

HK4, which is the only HK isoform that does not show any auto-inhibition by its product G6P (Salani *et al.* 2013). Glycolysis and mitochondrial oxidative phosphorylation are targets of biguanides.

Recent findings have shown that these compounds act more effectively in cellular models with defects in glucose utilization and mitochondrial function. For instance, mitochondrial DNA mutations in complex 1 genes are the determinants of cancer cell's sensitivity to phenformin when cells are grown with low glucose levels (Birsoy *et al.* 2014). *Vice versa*, high glucose concentrations reduce the effectiveness of metformin on breast cancer cells' proliferation (Wahdan-Alaswad *et al.* 2013). All these findings indicate that the interaction between glucose dose and metformin efficacy could have implications for the clinical use of metformin (Wahdan-Alaswad *et al.* 2013, Zordoky *et al.* 2014). In particular, the maintenance of glucose homeostasis could represent an important requirement for the clinical use of metformin for oncological treatments.

Insulin action and metformin: a metabolic point of view

Although several studies have shown that metformin has a direct effect on proliferation of tumour cells in diabetic patients, the inhibitory effect of metformin on cancer progression may be indirect through decreased insulin concentrations. Insulin may favour cancer cell proliferation and mitogenic processes (Cohen & LeRoith 2012). The gene encoding the insulin receptor (IR), by alternative splicing of exon 11, yields two isoforms: IR-A and IR-B. IR-A is principally overexpressed in cancer cells and differently from IR-B drives the mitogenic and proliferative pathways. IR-A and IR-B can form hybrid receptors activating the mitogenic pathway (Frasca *et al.* 2008). By binding to hybrid receptors, insulin stimulates specific IGF1 receptor (IGF1R) signalling pathways. In particular binding of insulin to insulin IR-A is the most accepted mechanism of mitogenic action of insulin. Insulin, even if with lower affinity, binds and activates IGF1R, which is expressed at high levels in tumour cells. Insulin decreases circulating concentrations of IGFBP1 and IGFBP2 (Jalving *et al.* 2010). This can improve the bioactivity of IGF1, which activates IGF1R and IR/IGF1R hybrid receptors with a similar potency (Slaaby *et al.* 2006). IR, IGF1R and IR/IGF1R phosphorylation activate the MAPK/ERK signalling pathway, stimulating cell growth and proliferation. Recently it has been demonstrated that MAPK/ERK can promote the Warburg effect, phosphorylating PKM2. PKM2 is one of the most important rate-limiting enzymes of glycolysis, which transfers phosphate from PEP to ADP generating ATP. MAPK/ERK induces Ser37 phosphorylation and nuclear translocation of PKM2. In the cytoplasm, PKM2 can exist as enzymatically active tetramer or as an inactive dimer. When a PKM2 dimer translocates

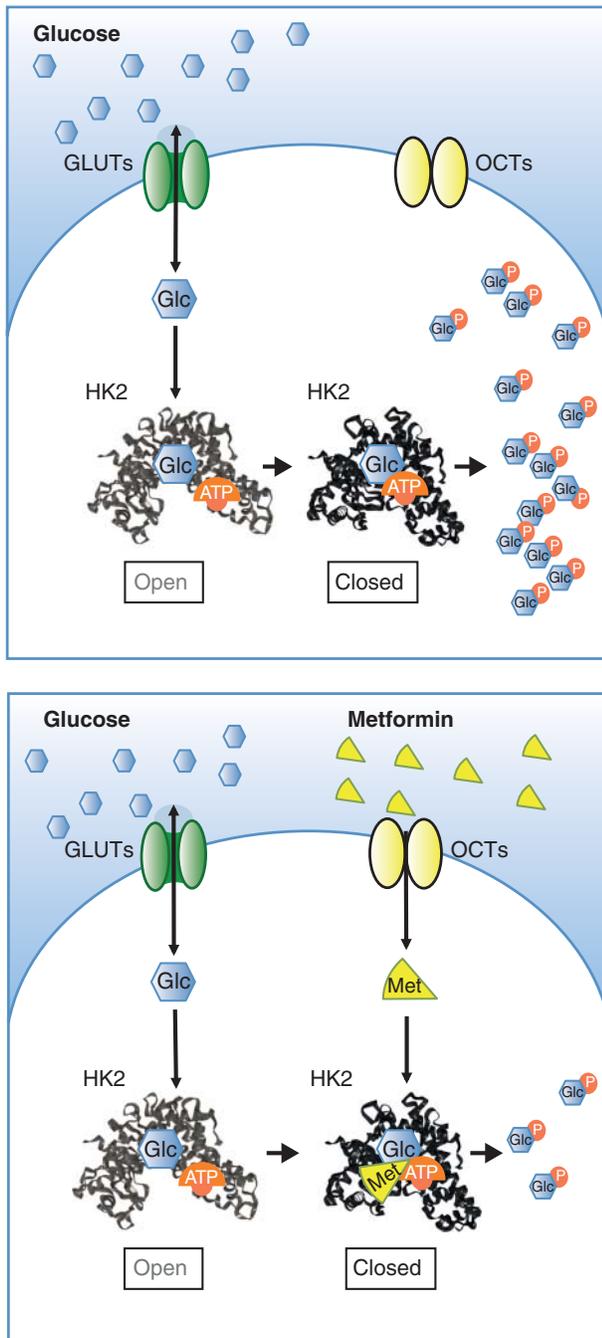


Figure 2

Metformin's inhibitory effect on HK2 activity. Binding of glucose to HK2 is required to induce conformational change in the enzyme from an open to a closed structure. Metformin inhibits HK2 only in the closed conformation. The region occupied by metformin corresponds to the binding site of G6P, an inhibitor of the enzymatic reaction.

into the nucleus, it becomes a potent protein kinase which phosphorylates histone H3 leading to transcription of genes, including cyclin D1 and MYC (Yang *et al.* 2012). The alternative PI3K/AKT signalling pathway drives cellular protection and energy metabolism. Indeed, IR and IGF1R pathways may act on biochemical intermediates of glycolysis, and recently it has been proposed that PI3K/AKT signalling pathway may have a role in cellular energy metabolism. AKT is a master antiapoptotic regulator, which can favour glycolysis and glucose uptake, inducing the expression of hypoxia-inducible factor 1 α and then of GLUT1 and HK2 in cancer cells (Semenza 2007). AKT phosphorylates HK2 at Thr473 and facilitates the association of HK2 with mitochondria, which is required not only to inhibit cytochrome *c* release and apoptosis but also to ensure the catalytic function of HK2 (Roberts *et al.* 2014).

In this scenario, the question is whether and how metformin could interfere with the increased cellular energy disposition induced by insulin and IGF1 in cancer cells. In mouse models, oral administration of metformin inhibits the mTOR pathway in normal lung epithelium and lung tumours by decreasing insulin and IGF1 concentrations. Metformin inhibits IGF1-induced AKT phosphorylation (Salani *et al.* 2012). Thus, taking into account the existence of a cross-talk between AKT and mitochondria/HK2, this finding opens the possibility that metformin could drive dissociation of HK2 from mitochondria not only by a direct inhibitory effect but also by an indirect inhibitory effect (Salani *et al.* 2013; Fig. 3).

Metformin's effect on glucose uptake: fluoro-deoxy-glucose imaging

The signal transduction pathways that regulate the Warburg effect during tumourigenesis remain important points to be explored. For this reason, metformin's ability to inhibit glucose metabolism in cancer cells may offer a possible benefit by limiting energy resources and consequently affecting cancer cell proliferation and growth. One of the most relevant clinical uses of the Warburg effect relies on the fact that the high rate of deregulated glucose consumption can be visualised, non-invasively, for cancer diagnosis and staging. In fact, recent decades have witnessed an impressive growth of clinical application of positron emission tomography (PET) scanners due to their capability to map glucose consumption by the use of ^{18}F -fluoro-deoxy-glucose (FDG). This analogue enters the cell via the same facilitative transporters of glucose; it is then phosphorylated to FDG6P and remains

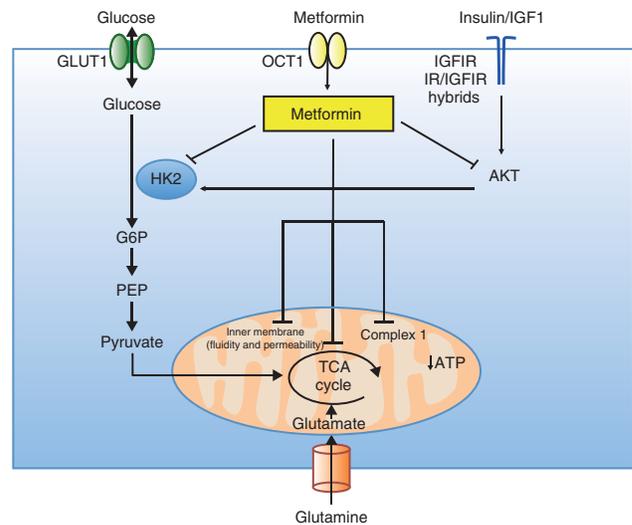


Figure 3

Proposed sites of action of metformin in cancer cells. The OCT1 transporter is highly expressed in cancer cells and mediates metformin uptake. Metformin's inhibitory action on glucose metabolism results from a combination of effects on glycolysis and growth factor signalling pathways. Metformin impairs glycolysis decreasing the enzymatic activity of the rate-limiting enzyme HK2. The inhibitory effect of metformin on AKT phosphorylation contributes to effects on HK2 by decreasing HK2 expression, activity and mitochondrial interaction. Metformin inhibits mitochondrial ATP production acting on respiratory chain complex 1, decreasing TCA cycle intermediates and modifying the inner mitochondrial membrane.

trapped within the cytosol, being a false substrate for all further reactions channelling G6P to alternative pathways. The evaluation of metformin's effect *in vitro* on glucose uptake has been carried out in different cancer cellular models (Fang *et al.* 2012, Birsoy *et al.* 2014). As AMPK negatively regulates glucose consumption in cancer cells (Faubert *et al.* 2013), the effect of metformin on AMPK phosphorylation was associated with a significant dose- and time-dependent reduction in FDG uptake. In a mouse model of prostate cancer, it has been shown that the decreased glucose oxidation exerted by metformin induces the dependency on reductive glutamine metabolism, which is an important source of cellular energy (Fendt *et al.* 2013). Glutamine is a substrate in the production of biochemical intermediates for the tricarboxylic acid (TCA) cycle. This anaplerotic function makes glutamine an important fuel and explains why several cancer cell lines consume glutamine at a higher rate than any other amino acid. Thus, interfering with glutamine metabolism may synergise with metformin's action in counteracting cancer growth and progression. Interestingly, in cancer stem cells (CSCs), metformin can deplete TCA cycle intermediates and alter the early metabolic state

of the neoplastic transformation processes (Janzer *et al.* 2014). To overcome this apparent contradiction, it is worthy of note that CSCs have distinct phenotypes from the cells forming the bulk of the tumour and, in fact, are detectably more chemo- and radio-resistant than differentiated cancer cells. Despite few studies reporting on the metabolic effects of metformin on CSCs, encouraging results appear to indicate its effectiveness on CSCs viability. In particular, metformin seems to have an effect chemoresistant CDC subpopulations (Hirsch *et al.* 2009), acting preferentially on CD133⁺ (Gou *et al.* 2013) and CD44^{high}/CD24^{low} cells (Zhu *et al.* 2014).

Owing to its peculiarity, the major advantage of FDG usage in clinical settings is linked with the short time needed to obtain a reliable estimate of glucose consumption. In fact, this technique allows early detection of the metabolic disturbance that is largely preceded by cell death. On one hand, this finding confirmed the robustness of the method, ruling out the possible contribution of cell loss to the low glucose consumption measured in cell cultures. On the other hand, it permitted elucidation the relevance of HK2 inhibition in cell ATP depletion, besides the well-known effect of metformin on mitochondrial respiratory complex 1. Also, usage of FDG confirmed the relevance of the glycolytic pathway in the maintenance of cancer growth and viability.

The extension of this model *in vivo* profoundly extended our understanding of the mechanism of action of metformin, documenting the role of indirect effects (caused by the modified whole-body metabolic patterns and signalling) and of direct actions on cancer glycolytic rate. The first evidence of metformin's effect in experimental cancer indicated that metformin can interfere with the diagnostic performance of FDG imaging in cancer detection and staging.

The tracer uptake in the different tissues is influenced by three main factors: i) lesion glucose consumption; ii) tracer availability in the blood; and iii) ratio between tracer and glucose plasma concentrations. Metformin markedly interferes with the latter two variables because its euglycemic action influences whole-body glucose disposal and thus FDG blood concentration. Moreover, this 'systemic tracer sequestration' is further amplified by the well-know increase in FDG uptake induced by metformin on colonic epithelium (Massollo *et al.* 2013), which further reduces tracer availability for cancer, independently from its own glucose consumption.

These considerations indicate that effect of metformin on cancer FDG uptake is related directly to the drug action on cancer metabolism and inversely to drug action on

whole-body glucose disposal. The simple measurement of lesion tracer retention (the so-called SUV) might thus represent a suboptimal index of the responses of lesions to the drug. To overcome these limitations, the metabolic effect of metformin has been recently tested in a model of triple-negative breast cancer. In that study, compartmental analysis (Marini *et al.* 2013) of FDG exchange between blood and cancer revealed a significant reduction in cancer glucose consumption that was not paralleled by a corresponding decrease in lesion SUV (Figure S1, see section on supplementary data given at the end of this article). Altogether, these data thus confirmed that the inhibitory effect on tumour glucose consumption contributes to the anticancer potential of metformin.

Drug development

Biguanides (Fig. 1) offer a combination of unique chemical features allowing the possibility of easy ionization and tautomerization and thus constituting a privileged pharmacophore-interacting moiety with a drug-like scaffold suggestive of *in vivo* pharmacokinetics (Sundriyal *et al.* 2008). Among biguanides, metformin can be considered as a particular case because it is a small and highly hydrophilic molecule, which is not bound to plasma proteins, is readily filtered at the glomerulus and is poorly resorbed due to its low lipid solubility, overall resulting in high renal clearance. Thus, oral absorption, hepatic uptake and renal excretion of metformin are mediated by OTCs, reflecting the distribution, elimination and biochemical effects of metformin in tissues and subcellular compartments (Graham *et al.* 2011). As other biguanides have been already demonstrated to encompass a unique set of pharmacophore features conferring the ability to adapt to different biological targets, pharmacological effects can be conceivably diverse and adaptable to different tautomeric forms (Sundriyal *et al.* 2008). While this behaviour contributes to the puzzling polypharmacological role of metformin, several studies have recently contributed to uncovering mechanistic insights into key enzymes involved in the glycolytic pathway (Da Silva *et al.* 2010, Salani *et al.* 2013). For instance, as mentioned above, metformin is able to directly inhibit HK1 and HK2, and molecular modelling results support the idea of an allosteric inhibition, which raises the possibility of further chemical optimisations of the binding of the biguanide scaffold into the HK1/HK2 binding sites. Thus, biguanides can be considered as useful starting points for drug discovery purposes, embracing therapeutic indications going beyond the treatment of

type 2 diabetes, in particular, for cancer diseases (Martin & Marais 2012). It should be noted that, while scientific data demonstrate the potential beneficial effects of metformin on several cancer types, a thorough assessment of the structure–activity relationship of other biguanides is yet to be demonstrated. This consideration is relevant to creation of a link with specific biological targets – including HKs – and, in the long run, will be essential for embarking on new clinical trials based on the use of metformin or other biguanides (Suissa & Azoulay 2012). It should be noted also that, because the expression of OCTs may differ between various tumour types, the direct action of metformin may be critically limited where expression of OCTs is low, while it could be greatly improved by conceiving new biguanide derivatives, or by repurposing known drugs such as phenformin or buformin. These drugs have been dismissed in the past due to the onset of severe lactic acidosis, but the mechanism underlying this side effect remains elusive. In addition, this risk might be not be contraindicated for dosing in cancer treatments, especially if the superior activity of these agents is clearly demonstrated (Sogame *et al.* 2013). Importantly, in the context of the development of new biguanide-based compounds, drug discovery techniques that include computer-aided molecular design tools such as molecular docking or pharmacophore screening, are expected to support the conception of new molecular entities with improved biological profiles (Del Rio *et al.* 2010, Caporuscio *et al.* 2011, Sanders *et al.* 2012).

Perspectives

Metformin is a first-line anti-diabetic drug showing interesting anticancer effects. In diabetic patients, it inhibits hepatic glucose production, by reducing cellular concentrations of ATP and then mimicking a fasting condition. To be responsive, metformin cells must express its facilitative transporter (OCT1). Several cancer cells express both OCT1 and HK2, making them responsive to the toxic action of metformin. In contrast, the prevalent expression of OCT1 and HK4, not inhibited by metformin, in the liver and the combined low OCT1 expression irrespective of the presence of HK2, in other tissues, allow its use in the prevention of massive cell death. *In silico* modelling has provide clues about this inhibitory mechanism opening avenues for investigating other HK2 inhibitors. These new compounds should still be dependent on OCT1 transport into cancer cells, preserving normal cells and representing an ideal anticancer treatment to kill cancer cells without affecting other tissues.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/ERC-14-0284>.

Declaration of interest

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

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