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Metformin and Pancreatic Cancer Metabolism

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1. Introduction

Numerous epidemiological studies have reported that metformin, a well-known and widely used anti-diabetic drug, may provide protective benefits in decreasing pancreatic cancer risk among the diabetic population. Following a brief introduction regarding metformin's history and pharmacological properties, this book chapter presents epidemiological findings showing how metformin is associated with protection against pancreatic cancer. We also introduce the anti-cancer effects of metformin through AMPK-independent and AMPK-dependent manners [1-6]. These mechanisms include its inhibitory effects on the insulin growth factor-1 (IGF-1), G protein-coupled receptor (GPCR) and mTORC1 signaling pathways [3-10]. We then discuss the metabolic effects of metformin in cancer. For example, metformin has been shown to inhibit glycolysis in various cancer cell lines [11-13]. Metformin is a known inhibitor of complex I of the electron transport chain [14-18], potentially limiting the intact oxidative respiration capabilities of the cancer cell. We also discuss in depth the anti-cancer mechanisms of action of metformin in the context of lipid metabolism as reported in numerous models. These include metformin's ability to increase fatty acid β -oxidation in adipocytes [19] and its ability to inhibit hepatic lipogenesis [2]. As shown by numerous studies [20-23], metformin also possesses anti-lipogenic properties, potentially limiting this critical metabolic pathway that confers cancer pancreatic cell survival advantage.

We provide preclinical and clinical evidences of the potential utility of metformin in pancreatic cancer. For example, a very recent report has shown tumor growth inhibition *in vitro* and *in vivo* by metformin through down-regulation of Sp (specificity protein) transcription factors and consequent down-regulation of the Sp-regulated genes.[24]. Metformin has been also shown to impair tumor development in pancreatic cancer in xenografts models [25]. Finally, we also explore the role of lipid metabolism in the context-specific ability of metformin to act as a chemopreventive/therapeutic agent. As early as 2001, it has been reported that metformin significantly impairs the formation of pancreatic lesions induced by the pancreatic carcinogen

N-nitrosobis-(2-oxopropyl)amine in hamsters fed a high fat diet [26]. We will argue that the in order for metformin to exert its anti-cancer properties, consideration of the genetic and metabolic status of the model system is critical.

We conclude this chapter by discussing our most recent findings that show how metformin inhibits glucose-derived fatty acid synthesis in the context of available acetyl-CoA and the presence of *K-ras* mutation in pancreatic cancer cells in the context of obesity, the metabolic syndrome and diabetes [21]. These results strongly suggest that metformin, being an anti-lipogenic drug, may be useful when combined with lipid lowering and chemotherapeutic agents. Finally, because up-regulation of fatty acid synthase (FAS), the enzyme that catalyzes the terminal step in palmitate synthesis, is associated with increased resistance to gemcitabine and radiation treatments in human pancreatic cancer tissues [27], we argue that the use of metformin could synergize with these treatments.

However, an important question remains on whether or not metformin really has chemopreventive and/or therapeutic use for pancreatic cancer. This chapter argues that metformin does have anti-cancer properties by examining numerous experimental studies on metformin's potential mechanisms of action along with the metabolic and genetic context by which metformin may act as an anti-cancer drug.

2. The history behind metformin

Galega officinalis (also known as the French lilac or Goat's Rue) is a plant that has been used for the treatment of *diabetes mellitus* in traditional medicine for centuries. At the end of the 19th century, guanidine compounds were discovered in *Galega* extracts (Figure 1). Shortly after, in 1918 animal studies showed that these compounds lowered blood glucose levels [28]. However, guanidines are fairly toxic. Following this discovery, some less toxic derivatives, named synthalin A and synthalin B (Figure 1), were synthesized based on the structure of galegine and used for diabetes treatment under the marketed name of Synthalin in the 1940s. The discovery of insulin overshadowed the use and further development of synthalin compounds and they were forgotten for the next several decades. When chemists found that they could also make the guanidine compounds more tolerable by bonding two guanidines together, forming a biguanide, interest in these molecules was regained and attention was focused on metformin, phenformin and buformin (Figure 1). Finally, the interest in metformin, synthesized by K. Slotta, was further renewed in the late 1950s after several reports that it could reduce blood sugar levels in people. The French physician Jean Sterne published the first clinical trial of metformin as a treatment for diabetes in 1957 [29].

3. Synthesis, structure and pharmacology of metformin

Synthesis: Metformin is synthesized by equimolar fusion of hydrochloric dimethylamine and dicyandiamide at 130-150°C for 0.5 to 2 hours [30].

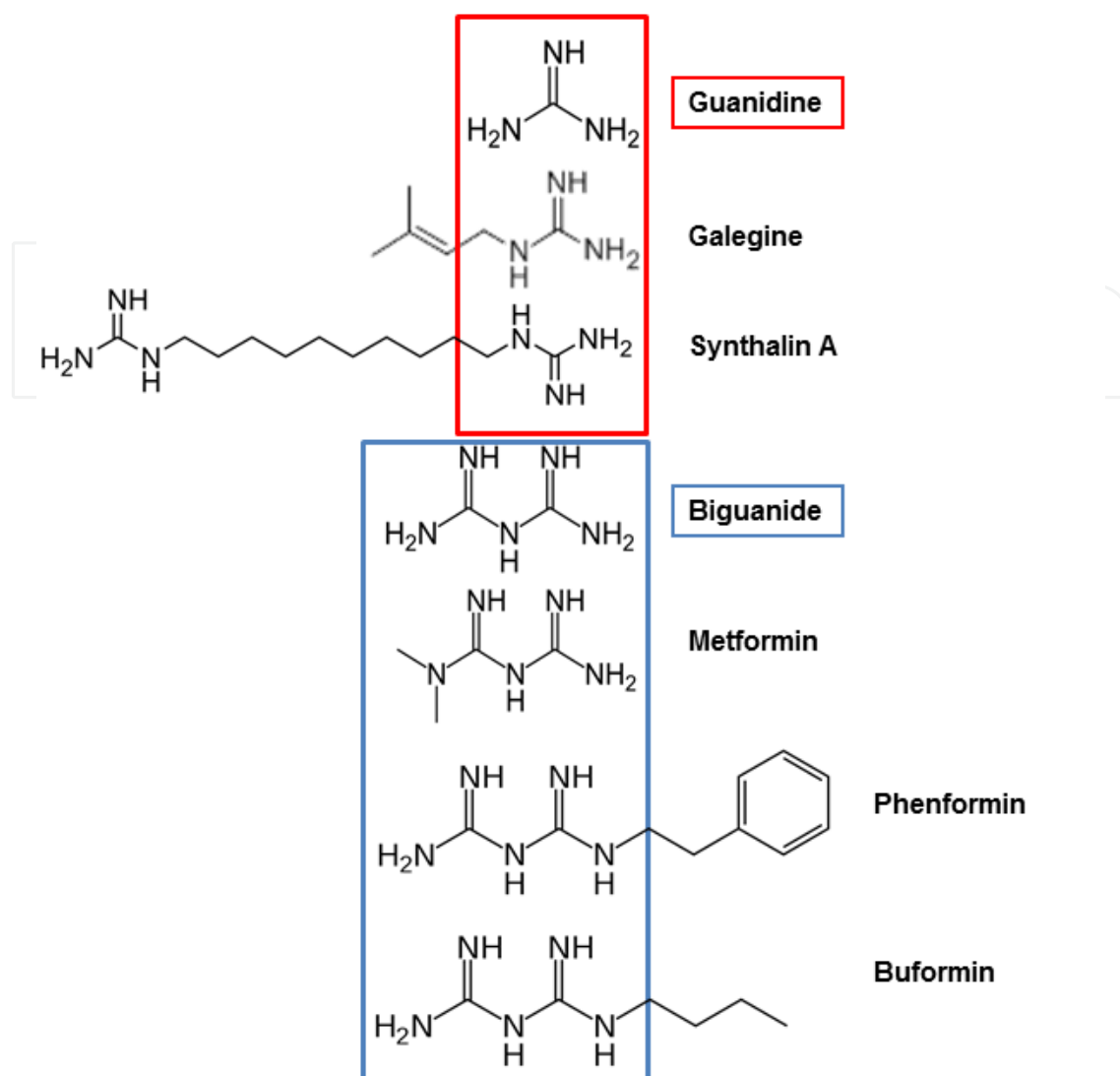


Figure 1. Structures of guanidine and biguanides.

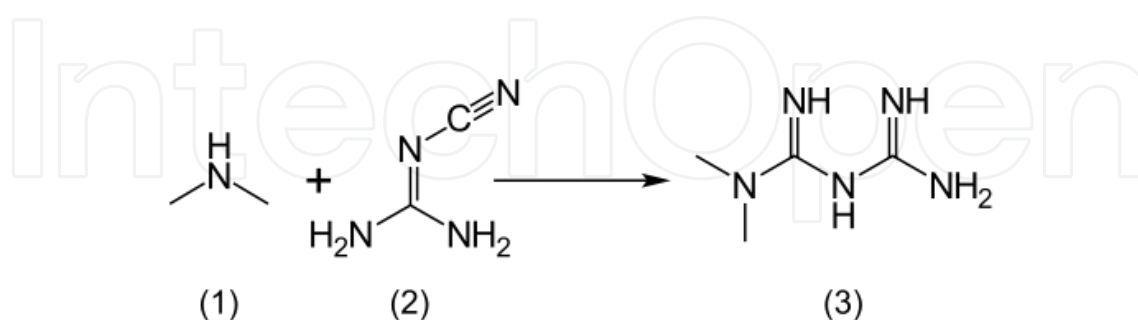


Figure 2. Synthesis of N,N'-dimethyl biguanide.

Structure: Metformin is globally charged positively and does not or poorly permeates the plasma membrane. The structure was represented in a wrong tautomeric form until it was corrected in 2005 by a group of chemists from India [31]. Metformin is given to patients as a

hydrochloride from. Several studies have demonstrated an affinity of biguanides for phospholipids at the plasma membrane [32] as well as some protein binding [33]. The interaction of the biguanide with the polar head group of phospholipids induces a diminution of the plasma membrane fluidity leading to the rigidification of the plasma membrane [32]. However, this reduction of the fluidity has not been reproduced by Wiernsperger and collaborators [33] who showed an increase in fluidity of red blood cells membranes.

Metformin has an oral bioavailability of 50-60% under fasting conditions and the peak plasma concentration is reached within a couple of hours. The plasma protein binding is negligible. Metformin is not metabolized but it is accumulated in tissues such as the liver, the kidneys, the salivary glands and the gastrointestinal tract [34]. Eighty percent of the elimination of metformin occurs by the urinary tract. The average elimination half-life in plasma is 6.2 hours. The half-life of biguanides is approximately 2 hours [34]. Interestingly, metformin is distributed to (and appears to accumulate in) red blood cells with a much longer elimination half-life: 17.6 hours.

4. Chemopreventive properties of metformin

Numerous observational studies show that metformin use, when compared against other diabetic agents such as insulin and sulfonylureas, decreases cancer risk and overall cancer mortality among the diabetic population. These protective associations have been reported across different cancer types and among various diabetic populations.

Table 1A summarizes epidemiological studies that show pancreatic cancer risk and cancer mortality associated with metformin use while Table 1B presents observational studies and clinical trials on overall cancer risk and mortality in relation to metformin use. While some studies show a reduction in pancreatic cancer [35-39] and overall cancer risk [37, 39-45] among diabetic metformin users, there are also studies that report no significant difference in cancer risk among diabetics who take metformin compared to patients who take other anti-diabetic treatments [36, 46-51]. These conflicting results may be explained by differences in the study population, the confounding factors accounted for during statistical analysis and the selected study design (e.g., cohort *versus* case-control). For example, patients who were prescribed metformin may generally have better glucose control compared to those prescribed insulin hence, the risk for future diseases such as cancer may be lower at baseline for metformin users versus diabetics treated with other modalities. As shown in Tables 1A and 1B, different studies account for different confounding factors which can largely influence the results of disease risk calculations. There is no standardized procedure for the selection of which potential confounding factors are to be included in a statistical model hence; this can lead to large variations among observational study outcomes. Overall, although these epidemiological studies are correlative in nature and hence, cannot establish causality between metformin use and cancer risk and mortality, they provide a biological basis to further explore whether metformin possesses chemopreventive and/or chemotherapeutic properties.

5. Chemotherapeutic properties of metformin

Experimental studies show that metformin possesses anti-cancer effects in various cancer types. As the metabolic effects of metformin are discussed in the section “Metformin as an Anti-lipogenic Drug,” figure 2 provides a summary of metformin’s effects exclusively on cancer signaling pathways. Overall, we present metformin’s effects on cancer cells as AMPK-dependent (pathways 2, 4, 5, 6 and 10) and independent (pathways 1, 3, 7- 9 and 11). Although there is an overlap between cell signaling and metabolic alterations due to metformin treatment (e.g., via ETC complex I and ATP production, AMPK/mTORC1 axis and metabolic control), metformin’s anti-cancer effects can be grouped into: a) inhibition of ATP and ROS production, b) inhibition of IRS-1/Akt/mTORC1 axis, c) anti-inflammatory effects, d) cell cycle arrest and e) inhibition of general transcription factors.

First Author/ Year (reference)	Location	Design	Outcome	Comparison	Risk (95% CI)	Confounding Factors
Oliveria et al., 2007 [46]	United States	Cohort	Pancreatic cancer risk	Metformin vs no Metformin	RR: 1.26 (0.80-1.99)	Age, gender, gastrectomy, chronic pancreatitis, deep venous thrombosis, dermatomyositis/ polymyositis, alcoholism, hepatitis B/C, history of polyps
Currie et al., 2009 [47]	United Kingdom	Cohort	Progression to pancreatic cancer	Sulfonylureas vs Metformin Metformin + Sulfonylureas vs Metformin Insulin-based therapies	HR: 4.95 (2.74-8.96) HR: 0.38 (0.13-1.12) HR: 4.63 (2.64-8.10)	Age, sex, smoking status, diagnosis of a previous cancer
Li et al., 2009 [35]	United States	Case- control	Pancreatic cancer risk	Metformin	OR: 0.38 (0.22-0.69)	Age, sex, race smoking, alcohol, BMI, family history of cancer, diabetes duration, use of insulin
Bodmer et al., 2011 [36]	United Kingdom	Case- control	Pancreatic cancer risk	Metformin (both sexes) Metformin (females only) Sulfonylureas Insulin	OR: 0.87 (0.59-1.29) OR: 0.43 (0.23-0.80) OR: 1.90 (1.32-2.74) OR: 2.29 (1.34-3.92)	BMI, smoking, alcohol consumption, diabetes duration
Ferrara et al., 2011 [48]	United States	Cohort	Pancreatic cancer risk	Metformin and pioglitazone	HR: 1.2 (1.0-1.5)	Age, ever use of other diabetes medications, year of cohort entry, sex, race/ethnicity, income, current

First Author/ Year (reference)	Location	Design	Outcome	Comparison	Risk (95% CI)	Confounding Factors
						smoking, baseline HbA1c, diabetes duration, new diabetes diagnosis, creatinine, and congestive heart failure
Liao et al., 2011 [49]	Taiwan	Cohort	Pancreatic cancer risk	Metformin	HR: 0.85 (0.39-1.89)	Crude/ Unadjusted
Lee et al., 2011 [37]	Taiwan	Cohort	Pancreatic cancer risk	Metformin vs. potential use of other oral anti-hyperglycemic medications	HR: 0.15 (0.03-0.79)	Age, gender, other oral anti-hyperglycemic medication, Charlson comorbidity index score, time-dependent metformin use
Morden et al., 2011 [50]	United States	Cohort	Pancreatic cancer risk	Metformin	HR: 1.25 (0.89-1.75)	Age category, race/ethnicity, diabetes complications, obesity diagnosis, oral estrogen use, Part D low income subsidy (a poverty indicator), 14 Charlson comorbidities, and tobacco exposure diagnosis
Ruiter et al., 2012 [38]	Netherlands	Cohort	Pancreatic cancer risk	Metformin vs. sulfonylureas	HR: 0.73 (0.66-0.80)	Age at first oral glucose-lowering drug (OGLD) prescription, sex, year in which the first OGLD prescription was dispensed, number of unique drugs used in the year, number of hospitalizations in the year before the start of OGLD
Sadeghi et al., 2012 [52]	United States	Cohort	Median survival in pancreatic cancer, prognostic factors of overall survival in pancreatic cancer	Metformin vs. Non-metformin Univariate Analysis: Metformin Multivariate Analysis: Metformin	15.2 months vs. 11.1 months (P = 0.009) HR: 0.68 (0.52-0.89) HR: 0.64 (0.48-0.86)	No significant differences in BMI, age, sex, race, diabetes duration, disease stage, tumor size, performance status, serum CA-19-9 between metformin and non-metformin group

First Author/ Year (reference)	Location	Design	Outcome	Comparison	Risk (95% CI)	Confounding Factors
Nakai et al., 2013 [51]	Japan	Cohort	Prognostic factors of overall survival in pancreatic cancer	Univariate Analysis: Biguanide Sulfonylureas Insulin Thiazolidine-dione	HR: 0.61 (0.19-1.44) HR: 0.60 (0.39-0.88) HR: 0.83 (0.59-1.15) HR: 0.19 (0.01-0.84)	Age (G65 or Q65 years old), sex (male or female), performance status (PS; 0Y1 or 2Y3), primary tumor size (G30 or Q30 mm), distant metastasis (yes or no), body mass index (G22 or Q22 kg/m ²), chemotherapy (combination therapy with gemcitabine and S-1 vs others), DM (yes or no), insulin (yes or no), sulfonylurea (yes or no), biguanide (yes or no), thiazolidine (yes or no), hypertension (yes or no), ACEI or ARB (yes or no), Ca-blocker (yes or no), A-blocker (yes or no), and statin (yes or no)
Singh et al., 2013 [53]	Various	Meta-analysis	Pancreatic cancer risk	Metformin use	OR: 0.76 (0.57-1.03)	
Zhang et al., 2013 [39]	Various	Meta-analysis	Cancer incidence and mortality	Metformin (and in combination with other drugs) vs. non-users	SRR: Incidence 0.54 (0.35-0.83) Mortality 0.64 (0.48-0.86)	

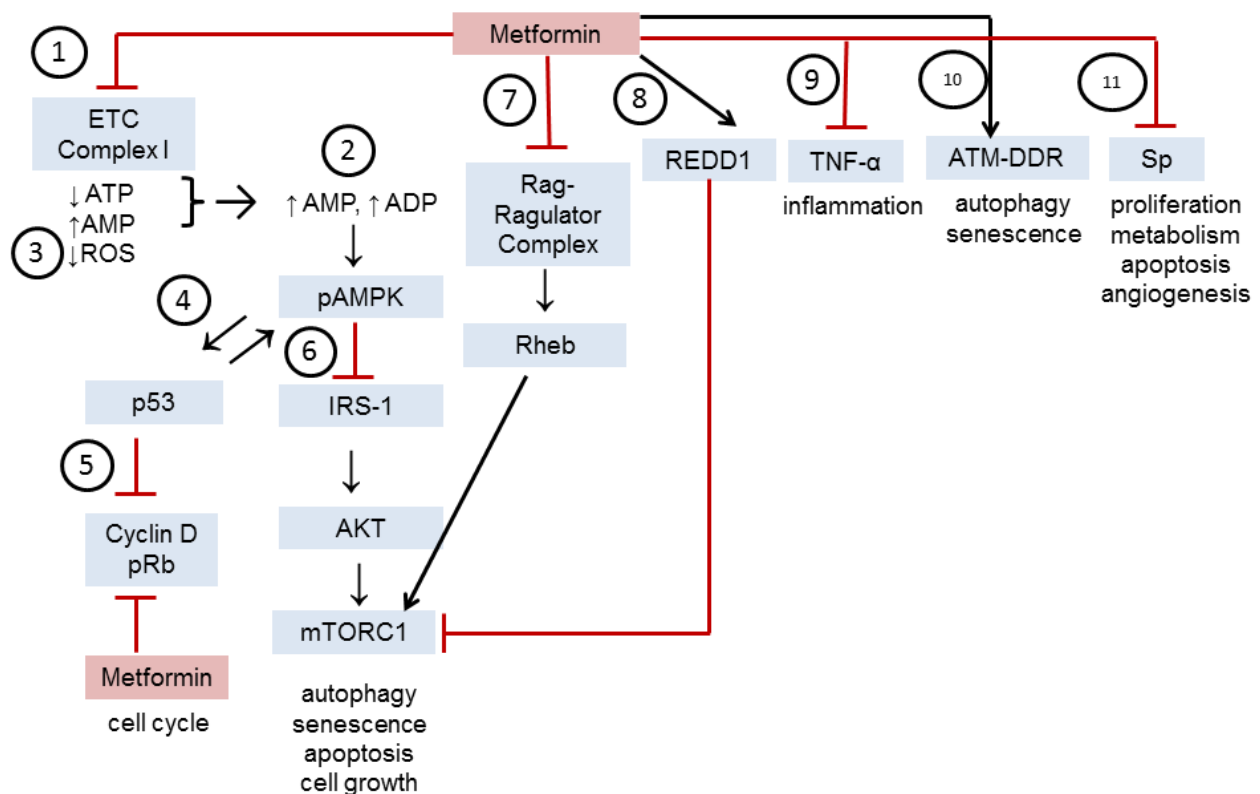
(a)

Bold type under "Risk" column indicates statistical significance using 95% confidence interval. HR, hazard ratio; OR, odds ratio, SRR, summary relative risk.

First Author/ Year	Location	Design	Outcome	Comparison	Relative Risk (95% CI)	Confounding Factors Accounted for
Evans et al., 2005 [41]	Scotland	Case-control	Cancer incidence	Metformin vs. no metformin	OR: 0.77 (0.64-0.92)	Smoking, body mass index, blood pressure, and postcode rank for material deprivation
Libby et al., 2009 [40]	Scotland	Nested case-control	Cancer deaths	Metformin vs. no metformin	HR: 0.63 (0.53-0.75)	Sex, age, BMI, A1C, deprivation, smoking, other drug use
Monami et al., 2009 [42]	Italy	Case-control	Cancer incidence	36 mo metformin vs no metformin	OR: 0.28 (0.13-0.57)	Concomitant therapies, exposure to

First Author/ Year (reference)	Location	Design	Outcome	Comparison	Risk (95% CI)	Confounding Factors
						metformin and gliclazide
Bowker et al., 2010 [44]	Canada	Cohort	Cancer death	Metformin vs. sulfonylurea	HR: 0.80 (0.65-0.98)	Age, sex and chronic disease score
Home et al., 2010 [54]	Various	Randomize d control trials	Cancer incidence	Metformin vs. rosiglitazone Metformin vs. glibenclamide Metformin + sulfonylurea vs. Rosiglitazone + sulfonylurea	HR: 0.92 (0.63-1.35) HR: 0.78 (0.53-1.14) HR: 1.22 (0.86-1.74)	Not reported
Landman et al., 2010 [45]	Netherlands	Cohort	Cancer mortality	Metformin vs. no metformin	HR: 0.43 (0.23-0.80)	Smoking (yes or no), age, sex, diabetes duration, A1C, serum creatinine, BMI, blood pressure, total cholesterol-to-HDL ratio, albuminuria, insulin use, sulfonylurea use, and macrovascular complications (yes or no)
Lee et al., 2011 [37]	Taiwan	Cohort	Cancer incidence	Metformin vs. no metformin	HR: 0.12 (0.08-0.19)	age, gender, other oral anti-hyperglycemic medication usage, CCI score and dose and duration of metformin exposure
Monami et al., 2011 [43]	Italy	Case- control	Cancer incidence	Metformin vs. no metformin in patients under insulin treatment,	OR: 0.46 (0.25-0.85)	Charlson comorbidity score (CCS), glargine mean daily dose (MDD), and total MDD of insulin
Zhang et al., 2013 [39]	Various	Meta- analysis	Cancer incidence and mortality	Metformin (and in combination with other drugs) vs. non- users	SRR: Incidence 0.73 (0.64-0.83) Mortality 0.82 (0.76-0.89)	
(b)						
Bold type under "Risk" column indicates statistical significance using 95% confidence interval. HR, hazard ratio; OR, odds ratio, SRR, summary relative risk.						

Table 1. A. Human Studies on Pancreatic Cancer Risk and Mortality with Metformin Use Among Diabetics, B. Human Studies on Overall Cancer Risk and Mortality with Metformin Use Among Diabetics



1) Metformin is a known inhibitor of complex I of the electron transport chain (ETC) [14, 15]. 2) The resulting decrease in adenosine triphosphate (ATP) production and increase in adenosine monophosphate (AMP) levels activate the kinase AMP-activated protein kinase (AMPK), a regulator of cellular energy status. Besides inhibition of energy-consuming biosynthetic processes (e.g., lipid synthesis and gluconeogenesis) and up-regulation of energy-generating catabolic metabolic pathways (e.g., β -oxidation of fatty acids and glycolysis), AMPK also signals to numerous proteins involved in cell survival, senescence, autophagy and death. For example, both high AMP and adenosine diphosphate (ADP) levels (from ETC complex I inhibition) is permissive for AMPK activation. AMP promotes AMPK phosphorylation at its catalytic α -subunit (Thr-172) by its upstream kinases liver kinase B1 (LKB1) and calcium/calmodulin-dependent protein kinase kinase-beta (CaMKK β), its allosteric activation and prevents dephosphorylation by protein phosphatase type 2a (PP2a) and protein type 2c (PP2c) phosphatases [55-57]. ADP also protects AMPK from dephosphorylation [58]. 3) The metformin-induced decline in endogenous reactive oxygen species (ROS) levels has been implicated to be involved in cancer risk reduction owing to its ability to reduce ROS-induced DNA damage [59]. 4) AMPK has also been shown to activate the tumor suppressor protein 53 (p53) (Ser-15) in inducing cancer cell cycle arrest and senescence [60]. The reversible arrows between p53 and pAMPK indicate that p53 has been shown to increase AMPK activity which, ultimately leads to mammalian target of rapamycin (mTOR) inhibition *in vitro* [61]. 5) Metformin has been shown to cause a G0/G1 cell cycle arrest by decreasing the expression of cyclin D1 and preventing the phosphorylation of pRb and hence, its inactivation [62]. 6) Metformin-induced AMPK activation has been shown to phosphorylate insulin receptor substrate-1 (IRS-1) at Ser-794 which results in decreased recruitment of the p85 subunit of phosphoinositide-3-kinase (PI3K), thus, impairing the insulin-like growth factor (IGF)-stimulated PI3K/protein kinase B/ mammalian target of rapamycin complex 1 (PI3K/Akt/mTORC1) signaling pathway [63]. Metformin has also been shown to inhibit the crosstalk between the insulin/IGF receptor and G protein-coupled receptor (GPCR) signaling, resulting in the inhibition of mTORC1 [4, 5]. 7) Biguanides are implicated in the inhibition of the Rag-dependent mTORC1 signaling [8], by preventing the co-localization of mTORC1 with its activator Ras homolog enriched in brain (Rheb). Rags are GTPases comprised of four proteins RagA, RagB, RagC and RagD that heterodimerize to activate mTORC1 upon amino acid stimulation [10]. Rags bind to the Ragulator complex made up of mitogen-activated protein kinase scaffold protein 1 (MP1), p14 and p18 trimeric proteins, localizing mTORC1 from the perinuclear compartment (where Rheb is located) into the cytoplasm, preventing Rheb activation of mTORC1 [64]. 8) Metformin also increases the expression of the mTOR inhibitor, regulated in development and DNA damage responses (REDD1), consequently down-regulating mTOR signaling [65]. 9) In human monocytes, metformin prevents lipopolysaccharide (LPS) and oxidized low density lipoprotein (LDL)-induced tumor necrosis factor (TNF) production at micromolar concentrations [66]. 10) Activation

and phosphorylation of AMPK is dependent on the serine-threonine kinase, ataxia telangiectasia mutated (ATM), a checkpoint that responds to double-strand breaks and oxidative stress by activating the DNA damage response involving numerous downstream targets such as p53, checkpoint kinase 2 (Chk2), breast cancer 1 (BRCA1), Fanconi anemia, complementation group 2 (FANCD2), Nijmegen breakage syndrome 1 (Nbs1), p53 upregulated modulator of apoptosis (Puma) - Phorbol-12-myristate-13-acetate-induced protein 1 (Noxa) and BCL2-associated X protein (Bax) [67, 68] thus, preventing further DNA insult. 11) Metformin induces nuclear degradation and decreased expression of Sp proteins, transcription factors for genes involved in cell proliferation (cyclin D1), metabolism (FAS), apoptosis ((B-cell lymphoma 2 (bcl-2) and survivin)) and angiogenesis ((vascular endothelial growth factor (VEGF) and its receptor VEGFR1)) [24].

Figure 3. Metformin impairs signaling molecules for cancer survival.

6. Overall physiological and cellular effects of metformin in cancer models

Contrary to sulfonylureas, which act at the level of the pancreatic secretion of insulin, biguanides act at the level of sensitivity of the target tissues for insulin. Moreover, the biguanides can reduce the hyperglycemia without leading to incidental hypoglycemia. Hence, the term “anti-glycemic” agent was coined for metformin.

In the late 90s, amongst many studies published on the cellular effects of metformin, we showed that metformin is able to modulate the insulin receptor (IR) in cholesterol (chol)-treated human hepatoma cells, HepG2 [69]. In that study, we used a cellular model in which insulin sensitivity was altered by supplementing the culture medium of HepG2 cells with a derivative of CHOL, cholesteryl hemisuccinate (CHS) [70, 71]. Overall, metformin did not affect IR phosphorylation in control cells. However, metformin affected IR autophosphorylation in CHS-treated cells. At 1 and 5 min of insulin stimulation, metformin increased IR phosphorylation in these cells, restoring IR phosphorylation in CHS-treated cells towards control levels. As mentioned earlier, metformin is a charged biguanide, requiring cell surface transport protein for its influx [72] and exhibits membrane effects as well as cellular effects [73]. Pertinent to our early work, recent studies from Algire et al. [74] demonstrated that a high energy diet promotes tumor growth and that metformin decreases tumor volume only in high-energy fed animals. The authors suggest that, “*the inhibitory effect of metformin on tumor growth was restricted to animals on the high-energy diet. These results suggested that any benefits of this drug in reducing cancer aggressiveness may be restricted to a metabolically defined subset of cancer patients.*” [74].

After nearly two decades of research and approval of metformin by the FDA in 1994, the target of the compound has yet to be identified. Arguably, as mentioned above, metformin is a charged biguanide, requiring cell surface transport protein for its influx [75] and exhibits membrane effects as well as cellular effects [73]. While Algire et al found that the anti-tumor effect of metformin was limited in animals on high-energy diets using *in vivo* models of lung and colorectal xenografts [76]; very recently, the work from Rozengurt and Eibl (from UCLA) demonstrates a strong tumor growth delay effect of metformin in pancreatic cancer xenograft models [5]. However, the doses used (>200mg/kg, i.p.) may not be clinically relevant. The ongoing debate on metformin dosages in animal models and human clinical trials has yet to define clearly the anti-diabetic dose *versus* the anti-cancer dose as well as a preventive *versus*

a treatment dosage. The usual anti-diabetic dose of metformin is 500 - 1250 mg PO BID. Maximum recommended dose in patients with diabetes is 1250 mg PO BID. There seem to be some difference in data regarding prevention or actual treatment of cancer with metformin. The typical serum concentration from 1000- 2000 mg/day of metformin in diabetic patients is about 0.5 - 2 mg/L. The retrospective data so far have primarily looked at these doses and have shown that metformin prevents some cancers (including pancreatic cancer) [52, 77]. If we look at data regarding treatment of pancreatic cancer with metformin, it is different in terms of dosing. The pre-clinical data show that much higher doses are used (10-100 times the clinical used doses). If we look at data in humans using metformin for treatment of cancers, it shows some benefit at clinically used doses but it certainly is not as impressive as pre-clinical high dose metformin [78-80]. We did not find any ongoing human study using very high doses (beyond 3000 mg/day) primarily to treat cancers. Further studies need to be performed addressing this issue within the same animal model or within a similar patient population.

Finally, the fact that metformin prevents tumor development and growth in nude mice [5], supports a potential priming effect of metformin on the host potentially limiting the availability of 'onco' metabolites for which the tumor is 'addicted'. These types of studies investigating the processes of carcinogenesis, may address important gaps in current knowledge regarding the role of tumor metabolism in drug response. We strongly believe that mechanistic insight on these issues will have exceptionally high impact and potentially re-shape current paradigms about anti-metabolic drugs, pancreatic cancer treatment and personalized medicine. Indeed, we speculate that the efficacy of metformin – and possibly drugs with similar mechanism – depends on the metabolic context in which the tumor exists. This is potentially, a paradigm-changing concept as it suggests that host/tissue metabolic factors play a role in tumor conditioning and influence treatment response; a hypothesis that has not previously been considered in the clinical evaluation of metformin.

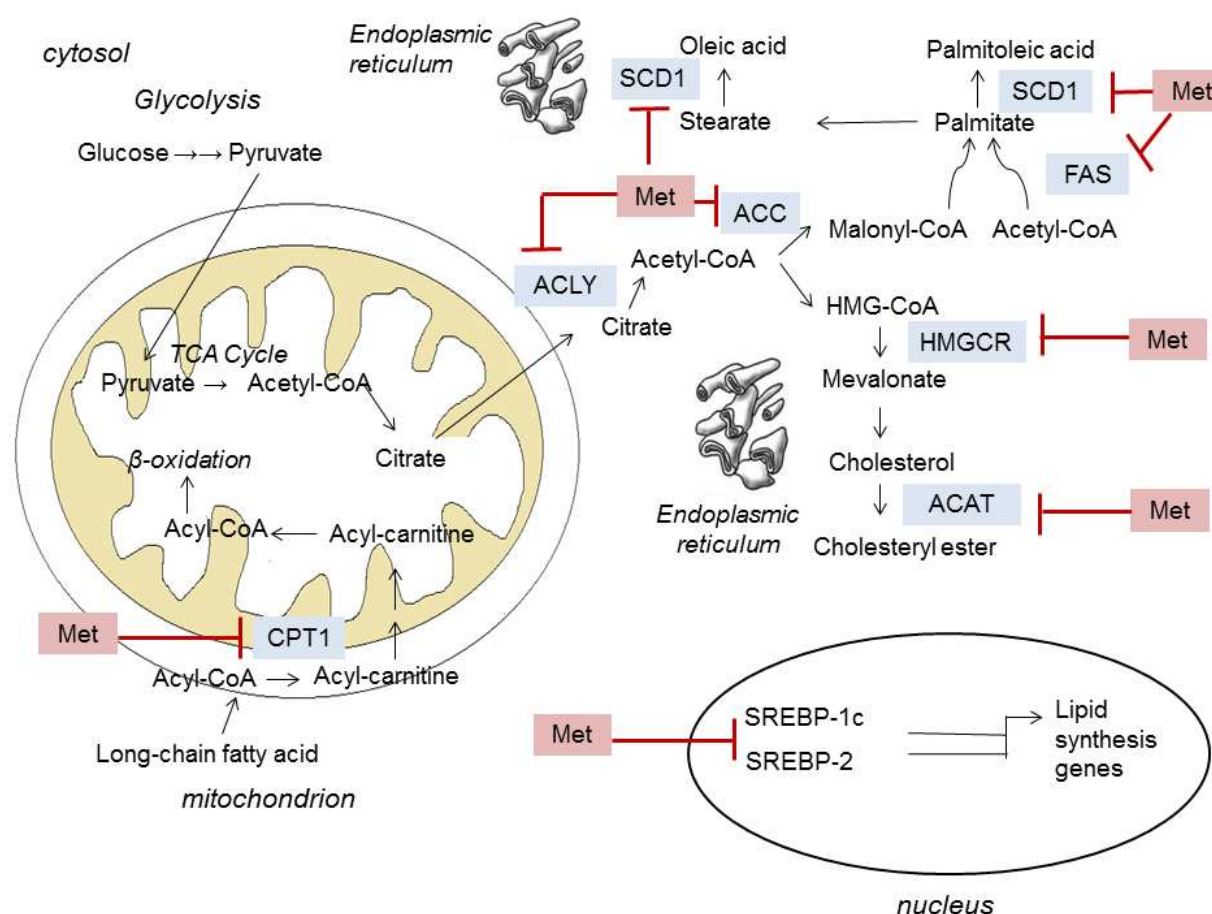
6.1. Metformin as a glucose lowering drug

Metformin works by decreasing hepatic gluconeogenesis [81], activating insulin receptor tyrosine phosphorylation [82], decreasing intestinal glucose absorption and increasing skeletal and adipose tissue glucose uptake [82]. One mechanistic study conducted in mice demonstrates that metformin (250 mg/kg/day for three consecutive days) increases the association of the glycolytic enzymes hexokinase to the mitochondria and phosphofructokinase to F-actin in mice hearts [83]. These associations result in their activation and up-regulation in glycolysis, increasing cardiac glucose utilization which may partly explain the cardio protective effects of the drug [84, 85]. Since 1995, metformin has been a widely prescribed glucose lowering agent in the United States for type 2 diabetic and polycystic ovary syndrome patients. It is a well-tolerated drug with lactic acidosis as a reported serious side effect [86]. However, the link between lactic acidosis and metformin use has recently been questioned [87].

6.2. Metformin as an anti-lipogenic drug

Dating back to the 1920's, Otto Warburg published his observations on the metabolic aberrations of cancer cells. In the seminal paper entitled "The Metabolism of Tumors in the Body,"

Warburg and colleagues showed the absence of lactic acid accumulation in the blood of normal animals (no cancer) [88]. Whereas, animals with tumors accrued greater concentration of lactic acid in venous compared to arterial blood as well as in the tumor cavity, indicating the formation of lactic acid from glucose fermentation as blood goes through the tumor [88].



Metformin inhibits the gene expression of carnitine palmitoyltransferase 1 (CPT1), a mitochondrial enzyme that is the rate-limiting step in long-chain fatty acid β -oxidation. CPT1 catalyzes the transfer of acyl-CoA to the carnitine hydroxyl group, forming acyl-carnitine which is then transported into the mitochondrial matrix via translocase. Carnitine palmitoyltransferase 2 (CPT2) catalyzes the formation of acyl-CoA from acyl-carnitine. Acyl-CoA then undergoes β -oxidation. Metformin prevents the nuclear activation of sterol-regulatory element-binding protein 1c isoform (SREBP-1c) and SREBP-2 sterol-regulatory element-binding protein 2 isoform (SREBP-2), transcription factors that induce the expression of enzymatic genes involved in fatty acid and cholesterol synthesis, respectively. Metformin decreases the activities of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR) and acyl coenzyme A:cholesterol acyltransferase (ACAT). HMGCR is the rate-limiting enzyme of the mevalonate pathway, that catalyzes the reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) to mevalonate. The mevalonate pathway synthesizes isoprenoids and cholesterol. ACAT is an endoplasmic reticulum protein that catalyzes the formation of cholesterol esters from acyl-CoA and cholesterol. Metformin decreases the gene expression of steroyl-CoA desaturase 1 (SCD1), the enzyme responsible for desaturation of stearic acid (18:0) into oleic acid (18:1 n-9) and of palmitic acid (16:0) to palmitoleic acid (16:1 n-7). MET decreases the protein expression of FAS, acetyl-CoA carboxylase (ACC) and ATP citrate lyase (ACLY), which are enzymes involved in fatty acid synthesis.

Figure 4. Metformin inhibits key metabolic steps in lipogenesis.

The reliance of cancer cells on glucose metabolism stems from their need to generate metabolites and reducing equivalents that are used to support crucial biosynthetic reactions that make lipids, nucleotides and amino acids. These biomolecules are rate-limiting for cell proliferation and survival. Glycolysis yields glucose-6-phosphate that enters the oxidative arm of the Pentose Phosphate Pathway (PPP). The oxidative PPP produces NADPH which, together with acetyl-CoA, fuels lipid synthesis in the cytosol. The non-oxidative branch of the PPP yields ribose-5-phosphate that is the precursor for nucleotides. In fact, as early as 1998, it has been argued that the both PPP branches (but primarily the non-oxidative branch) serve to produce ribose to sustain the increased needs of the cancer cell for DNA and RNA [89]. Fructose-6-phosphate and glyceraldehyde-3-phosphate are by-products of the glycolytic and the non-oxidative pentose phosphate pathways, providing an intimate link between glucose metabolism and nucleotide generation. Acetyl-CoA produced from the pyruvate dehydrogenase reaction enters the tricarboxylic acid (TCA) cycle in the mitochondria. Citrate can be exported from the mitochondria into the cytosol and converted back to acetyl-CoA (catalyzed by ATP citrate lyase) for lipid synthesis. Malate, an intermediate in the TCA cycle, can be converted into pyruvate by malic enzyme with the production of NADPH, a reducing equivalent that is used to generate reduced glutathione, allowing cancer cells greater tolerance to free radical-induced damage [90]. Glutaminase catalyses the hydrolysis of the amine group of glutamine to form glutamate and ammonia. Glutamate equilibrates with α -ketoglutarate via glutamate dehydrogenase. In a process termed reductive carboxylation, glutamine-derived citrate provides the acetyl-CoA for lipid synthesis and TCA cycle intermediates [91]. Hence, the glutamine addiction of cancer cells is another mechanism by which the metabolism is rewired to support biosynthesis [90, 91]. Please refer to Figure 3 for an integrated visual of cancer metabolism.

Diabetes and cancer are both metabolic diseases. It is therefore, not surprising that the mechanisms of action of metformin against type 2 diabetes and cancer include the drug's ability to alter critical metabolic circuits that lead to the normalization of blood glucose in diabetes and the impairment of biosynthetic pathways in cancer cells. For example, it is well-established that metformin is an inhibitor of complex I of the ETC. In 2000, two research groups have independently shown that dimethylbiguanide selectively blocks complex I of the ETC [14, 15]. In intact isolated hepatocytes, dimethylbiguanide has been reported to dose-dependently (0.1 to 10mM) inhibit oxygen consumption maximally at 20-30min [15]. The inhibition of respiration only occurred when glutamate-malate (complex I substrates) were used as substrates versus when succinate (complex II substrate)-rotenone or *N, N, N', N'*-tetramethyl-1,4-phenylenediamine dihydrochloride (TMPD)-ascorbate (complex IV substrates) were added during the assessment of oxygen uptake. It is interesting to note that El-Mir and colleagues did not observe these changes when oxygen uptake experiments were performed in digitonin-permeabilized hepatocytes or in isolated liver mitochondria [15]. This is in contradiction to what Owen and others published when they showed that lower metformin concentrations of 50 and 100 μ M were able to significantly decrease state 3 respiration rate in digitonin-permeabilized rat hepatoma cells [14]. Metformin has slow permeation properties across the inner mitochondrial membrane [14] and longer incubation periods (30 min in the El-Mir group versus 24-60 hours in the Owen group) may have eventually yielded comparable

results. In support of the previous findings, recent reports confirm that metformin is a specific inhibitor of the ETC complex I which leads to some impairment in mitochondrial function in human-derived non-malignant and in cancer cells [16-18, 92, 93]. This potentially limits the intact oxidative respiration capabilities of the cancer cell [16].

Besides inhibition of complex I and effects on glucose metabolism, numerous studies also show metformin-induced metabolic changes in non-cancer and cancer cells. One of the most notable effects of metformin is inhibition of lipogenesis, a metabolic pathway that is critical for a cancer cell's survival advantage. Under lipogenic conditions, surplus glucose in the cell is converted to pyruvate via glycolysis in the cytoplasm. Pyruvate is converted to acetyl-CoA and transported as citrate from the mitochondria into the cytoplasm. ATP citrate lyase (ACLY) converts citrate back to acetyl-CoA. Acetyl-CoA carboxylase (ACC) catalyzes the carboxylation of acetyl-CoA to malonyl-CoA in an ATP-dependent manner. Acetyl-CoA and malonyl-CoA are then used as substrates for the production of palmitate by the seven enzymatic reactions catalyzed by FAS. In cancer, *de novo* fatty acid (FA) synthesis is up-regulated mainly for membrane production (usage of FA for phospholipids) and post-translational modification of proteins [94]. ACLY, ACC and FAS expression and activity have been shown to be up-regulated in cancers including pancreatic cancer. Thus, metabolic enzymes involved in FA synthesis have emerged as therapeutic targets in cancer [94]. The effects of MET on energy homeostasis in normal hepatocytes and breast and colon cancer cells have been characterized by the blocked activation or expression of key lipid biosynthesis enzymes such as ACC, FAS, HMGCR and enhanced expression of regulators of mitochondrial biogenesis, peroxisome proliferator-activated receptor-gamma co-activator 1 (PGC-1) [1, 74, 95].

Suppression of anabolic pathways (metformin is anti-lipogenic) is in keeping with the expected consequences of AMPK activation [1]. HMGCR may also play an important role in human malignancies. Indeed, recent transcriptional profiling demonstrated that cholesterol and lipid metabolisms are linked to cellular transformation [96]. Interestingly, high HMGCR mRNA levels correlated with poor patient prognosis and reduced survival. The levels of additional mevalonate (MVA) pathway genes were also significantly correlated with poor prognosis of breast cancer patients, suggesting the entire pathway may be deregulated in these cases [97]. It is interesting to note that the metformin-induced inhibition of respiration is blocked by the addition of palmitate in 3T3-L1 adipocytes [19]. Adipocytes treated with palmitate complexed to albumin in the presence of carnitine had comparable oxygen consumption rates when compared to control. These results indicate that the metformin-induced inhibition of respiration can be reversed by the addition of fatty acids, which led the authors to conclude that the mechanism of action of metformin may be linked to fatty acid metabolism [19]. Although indirect, this article presented a link between metformin and its effects on lipid metabolism or *vice versa*.

The normoglycemic effects of metformin has also been attributed to its ability to prevent fatty acid oxidation which decreases acetyl-CoA, ATP and reducing equivalents' availability for hepatic gluconeogenesis [98], an effect likely mediated by a reduction in the expression of the carnitine palmitoyltransferase I gene [99] and eventually, a decrease in protein expression and

activity of the enzyme resulting in impairment in long fatty acid chain transport from the mitochondrial outer membrane into the matrix where β -oxidation takes place. Current publications also render support to the lipid-inhibitory effects of metformin. Metformin (0.2 to 1.0 mM for 16 h) has been shown to activate AMPK and decrease the mRNA, nuclear translocation and consequent activation via cleavage of the nuclear portion and the promoter activity of SREBP-1c in rat hepatoma McA-RH7777 cells [22, 23]. The mRNA and nuclear protein levels of SREBP-2 were also reduced after metformin treatment. This AMPK-mediated suppression of SREBP-1c has also been reported to prevent lipogenesis in an insulin resistant mouse model [20] and is consistent with a decrease in hepatic SREBP-1 expression in mice fed a high fat (60% lipids) diet for 10 weeks and then metformin (0.48mg% of the diet) for another six weeks [100]. Since SREBP-1c and SREBP-2 are transcription factors that promote the expression of enzymatic genes involved in fatty acid and cholesterol synthesis, respectively [101] we would expect diminished lipid synthesis as a biological endpoint of their down-regulation. In accordance, MRC5 human fetal lung fibroblasts incubated for 72 h with metformin (5×10^{-5} to 5×10^{-4} M) decreased $1\text{-}^{14}\text{C}$ acetate incorporation into sterols, fatty acids and triglycerides compared to control, accompanied by a reduction in the activities of HMGCR and ACAT, enzymes that catalyze the formation of mevalonic acid from HMGCoA and the esterification of cholesterol, respectively [102]. Also, metformin has been shown to induce the phosphorylation (Ser-351) of the nuclear receptor TR4 via AMPK, leading to decreased TR4 transactivation and a decrease in the gene expression of its target, steroyl-CoA desaturase 1 (*SCD1*) gene expression. SCD is an enzyme that catalyzes the synthesis of monounsaturated fatty acids palmitoleic acid (16:1 n-7) and oleic acid (18:1 n-9) from saturated fatty acids obtained from *de novo* lipogenesis or from the diet [103]. SCD1 has been shown to be associated to numerous diseases including but not limited to obesity, hepatic steatosis, hypertriglyceridemia, insulin resistance, low grade inflammation and bone fractures [103].

The role of SCD1 in cancer has been gaining more attention as a potential pharmacological target in cancer interventions [104]. SCD is an endoplasmic reticulum-bound protein encoded by the *SCD1* and *SCD5* genes in humans [105]. They are highly expressed in liver and adipose tissue (*SCD1*) and in the brain and the pancreas (*SCD5*) [103]. Observational studies have reported a positive association between saturation index (18:0 to 18:1 n-9 ratio used by investigators as a marker for SCD activity) with cancer risk [106-110]. The first cDNA of human SCD published in 1994 revealed that the mRNA levels of this enzyme were elevated in tissues derived from esophageal carcinoma, colorectal cancer and hepatocellular adenoma [111]. Its protein levels are highly expressed in SV40-transformed fibroblasts compared to their wild type counterpart [112]. However, decreased transcript expression was reported in prostate cancer when compared to normal epithelium [113]. These seemingly conflicting results may reflect variation in expression depending on the tissue type or some malignancy-induced metabolic changes in lipid synthesis and/or lipid profile which would overall guarantee cancer cell survival advantage. Indeed, Moore and others speculated that the down-regulation of SCD cDNA in prostate carcinoma may be due to: a) the need of the cancer cell to increase the levels of palmitate which can be done by decreasing SCD activity, b) eliminate the SCD-induced

down-regulation of lipid membrane rafts and c) down-regulate susceptibility of tumor cells containing more unsaturated fatty acids to TNF-induced free radical attack [113]. Nevertheless, knockdown of human SCD in transformed human fibroblasts resulted in decreased oleic acid synthesis, lowered desaturation index profiles of the main polar lipids phosphatidylcholine and phosphatidylethanolamine, and decreased *de novo* synthesis of ^{14}C -labeled phospholipids, cholesterol and cholesterol esters, free fatty acids and triacylglycerols [114]. Interestingly, there was an inhibition in cellular proliferation and anchorage-independent growth in the SCD knockdown cells [114]. Other studies confirm that SCD inhibition by chemical or genetic manipulations resulted in inhibition of cancer cell proliferation and/or death [115-118]. Thus, SCD appears to be involved in modulating lipid metabolism and signaling processes crucial for cancer cell replication and anchorage-independent growth, effects are likely influenced by the effects of SCD loss on membrane integrity [114].

In support of the anti-lipogenic effects of metformin, Bhalla and others [2] have reported that metformin decreased the gene and protein expression of enzymes involved in fatty acid synthesis namely, ACC, FAS and ACLY which was accompanied by a reduction in hepatic triglycerides in a mouse model of hepatocellular cancer fed metformin at a dose of 250 mg/kg for 24-36 weeks.

Obesity is a known risk factor for cancers of the pancreas, colon and rectum, esophagus, kidney, prostate, breast, uterus and ovaries [119-123]. In order to recapitulate this condition in the preclinical setting, animal models are fed high energy (HE), high fat (HF) diets to induce the metabolic syndrome and/or obesity. In an *in vivo* model of colon carcinoma by Algire and colleagues [124], metformin (50mg/kg/day for five weeks) significantly decreased tumor volume only in mice fed the HF/HE diet. Of note, these concentrations are more likely to be physiologically relevant to what a diabetic patient would have in their system. They also found that metformin reduced the expression of SREBP-1 and one of its target enzymes, FAS, regardless of the type of diet. Interestingly, a previous study from the same group using an *in vivo* model of lung cancer back in 2008 showed that the tumor growth inhibitory impact of metformin is exclusive to the mice under the HF/HE diet [76]. These two studies suggest that metformin may retard cancer growth depending on a particular metabolic state of the organism which in this case, is the abundance of circulating lipids from the HE/HF diet.

We recently found that the *in vitro* response to metformin depends on the level of intracellular cholesterol synthesis of the tumor [125]. We were the first to demonstrate that a physiologically relevant dose of metformin impairs glucose utilization in pancreatic cancer by inhibiting FAS when cholesterol synthesis is limited. Specifically, we found that pancreatic cells that have a *K-ras* mutation and that require *de novo* fatty acid (FA) synthesis for lipids ('lipogenic cells') were unable to synthesize FA from acetyl-CoA in the presence of an inhibitor of cholesterol synthesis and metformin. Our *in vitro* model shows that a physiologically relevant dose of metformin (100 μM) using an acute treatment of 24 h decreases *de novo* lipid synthesis via the FAS pathway in pancreatic adenocarcinoma only when: a) the glucose-derived acetyl-CoA is made available for fatty acid synthesis by inhibition of cholesterol synthesis (addition of exogenous cholesterol) and b) *K-ras* mutation is present [21]. As the fatty acid and cholesterol

synthesis pathways utilize acetyl-CoA as a common substrate [126], the addition of cholesterol (in the form of the more water-soluble cholesteryl hemisuccinate) inhibits the thiolase-catalyzed cholesterol pathway and shifts the glucose-derived acetyl CoA- towards the acetyl-CoA carboxylase-catalyzed fatty acid synthesis pathway. These effects are observed only in MiaPaCa-2-cells, which harbor the GGT(Gly) to TGT(Cys) codon 12 *K-ras* mutation. Further, non-lipogenic cancer cells harboring a *K-ras*^{G12C} mutation [127] with suppressed cholesterol synthesis were significantly more sensitive to the growth inhibiting effects of metformin than tumor cells containing wild-type *K-ras* with normal cholesterol synthesis. These results are consistent with expected modulation of AMPK [1] and/or mTOR [128].

Title	Recruitment	Results	Conditions	Interventions
Metformin Hydrochloride in Treating Patients With Pancreatic Cancer That Can be Removed by Surgery	Not yet recruiting	No Results Available	<i>Pancreatic Cancer:</i> Stage IA Stage IB Stage IIA Stage IIB	<i>Drug:</i> metformin hydrochloride <i>Other:</i> pharmacological study
Metformin Combined With Chemotherapy for Pancreatic Cancer	Recruiting	No Results Available	Locally Advanced Pancreatic Cancer/ Metastatic Pancreatic Cancer	<i>Drug:</i> gemcitabine, erlotinib, metformin, placebo
Metformin Plus Modified FOLFOX 6 in Metastatic Pancreatic Cancer	Recruiting	No Results Available	Acinar Cell Adenocarcinoma of the Pancreas, Duct Cell, Adenocarcinoma of the Pancreas, Recurrent Pancreatic Cancer, Stage IV Pancreatic Cancer	<i>Drug:</i> metformin hydrochloride, oxaliplatin, leucovorin calcium, fluorouracil <i>Other:</i> laboratory biomarker analysis
Combination Chemotherapy With or Without Metformin Hydrochloride in Treating Patients With Metastatic Pancreatic Cancer	Recruiting	No Results Available	Pancreatic Cancer	<i>Drug:</i> capecitabine, cisplatin, epirubicin, gemcitabine, metformin
Treatment of Patients With Advanced Pancreatic Cancer After Gemcitabine Failure	Recruiting	No Results Available	Pancreatic Adenocarcinoma Advanced or Metastatic	<i>Drug:</i> paclitaxel, metformin
Gemcitabine+Nab-paclitaxel and FOLFIRINOX and Molecular Profiling for Patients With Advanced Pancreatic Cancer	Recruiting	No Results Available	Stage IV Pancreatic Cancer	<i>Drug:</i> Gemcitabine, nab-paclitaxel, FOLFIRINOX, metformin <i>Genetic:</i> Immunohistochemistry (IHC) Analysis

Table 2. Ongoing Clinical Trials on Metformin and Pancreatic Cancer

In summary, metformin's anti-cancer properties rest on its ability to impair cancer cell lipogenesis, a critical mechanism by which cancer cells maintain their survival advantage over normal cells. Metformin is able to control lipogenesis through inhibition of the transcription factors SREBP-1 and SREBP-2, inhibition of activities and/or expression of enzymes involved in cholesterol and fatty acid synthesis. We have shown that metformin's anti-cancer role is effective in select metabolic phenotype and likely, a particulate cancer genotype. Thus, it is important to understand the metabolic context by which metformin exerts anti-cancer effects so that the correct patient population can be selected for therapeutic purposes.

7. Ongoing clinical trials on metformin as a chemotherapeutic drug for pancreatic cancer

There is considerable interest in the anti-tumor action of the commonly used anti-diabetic drug metformin for the treatment and management of patients with pancreatic cancer. Enthusiasm for metformin has been significantly strengthened by *in vitro* and *in vivo* experimental findings of potent anti-tumor activity of metformin at therapeutically safe doses. As a result, a number of early phase trials are now being conducted to assess the efficacy of metformin in combination with standard and experimental therapeutics in pancreatic cancer patients. Although there are numerous studies that show the cancer preventive and cancer therapeutic actions of metformin in preclinical models, there is a need to conduct adequately powered clinical trials on the therapeutic effects of metformin that include prognosis and survival markers. At the time that this book chapter was being written, there are six ongoing clinical trials specifically on pancreatic cancer and metformin from the ClinicalTrials.gov website (Table 2).

8. Conclusions and perspectives

Metformin is an inexpensive and well-tolerated drug and its utility as a chemopreventive and/or chemotherapeutic agent can be harnessed when we identify the drug's target/s, optimal dosage, and the correct patient sub-population who will benefit from metformin treatment. Until then, metformin remains the most widely prescribed anti-diabetic drug in the world with an unknown mechanism of action. In the era of targeted cancer therapy, one may cautiously link gene mutations and oncogenes up and down-regulation to cancer and involve metabolic phenotyping of the patient for better selection and truly personalized medicine.

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