Colorectal and Prostate Cancer Risk in Diabetes: Metformin, an Actor behind the Scene

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Abstract

Both diabetes and cancer are prevalent diseases whose incidence rates are increasing worldwide, especially in countries that are undergoing rapid industrialization changes. Apparently, lifestyle risk factors including diet, physical inactivity and obesity play pivotal, yet preventable, roles in the etiology of both diseases. Epidemiological studies provide strong evidence that subjects with diabetes are at significantly higher risk of developing many forms of cancer and especially solid tumors. In addition to pancreatic and breast cancer, the incidence of colorectal cancer and prostate cancer is increased in type 2 diabetes. While diabetes (type 2) and cancer share many risk factors, the biological links between the two diseases are poorly characterized. In this review, we highlight the mechanistic pathways that link diabetes to colorectal and prostate cancer and the use of Metformin, a diabetes drug, to prevent and/or treat colorectal and prostate cancer. We review the role of AMPK activation in autophagy, oxidative stress, inflammation, apoptosis, and cell cycle progression.

Key words: Diabetes, Colorectal Cancer, Prostate Cancer, Metformin, AMPK, mTOR

1. Introduction

The current worldwide diabetic epidemic is not only influencing the morbidity and mortality of the present generation, but with no doubt has a trans-generational epigenetic inheritance impact with increasing prevalence for cancer with Type 2 Diabetes Mellitus (T2DM) (1-3). The raised risk of cancer in T2DM (4-7), and with a demographic shift in age towards the over 65 years old, translates into considerable financial and social burden on finite health care budgets. The link between T2DM and raised incidence of colorectal cancer (CRC) and prostate cancer (PC), and mortality, has been related to a constellation of risk factors pertaining to metabolic syndromes, specifically insulin resistance, hyperinsulinemia, and hyperglycemia (8-11). A significant point to note here is that excessive plasma concentration of insulin and glucose correlate with accelerated aging (12). Hence, there is an urgent need for targeted therapy for comorbid diabetes and cancer. One such medication is the old workhorse for T2DM, metformin, which has generated considerable attention. Therefore, our review primarily focuses on metformin use in diabetics with either colorectal or prostate cancers. The properties and biological mechanisms of metformin are further discussed in relation to diabetes and cancer, followed by its applications in *in vitro*, *in vivo* and clinical studies.

2. Biological actions, pharmacokinetics and pharmacogenetics of metformin

The history of metformin, a biguanide derivative, dates back to the Middle-Ages, and its structural analogue galegine was isolated from *Galega officinalis* (goat's rue, French lilac, Italian fitch); a plant native to the Middle East that has been used for treatment of diabetes in Europe (13). Accumulating evidence shows beneficial survival effects of therapeutic intervention with metformin for cancer patients with T2DM (Fig. 1). Metformin, a cationic (hydrophilic base) drug, exerts its pleiotropic pharmacological effects beyond those of metabolic control (14), and includes favorable anti-inflammatory outcomes (15, 16).

Information on the pharmacological response to metformin requires an understanding of both its pharmacokinetics and genetic variation of the different transporters for the di-directional movement of metformin across plasma membranes (17) (Fig.2). Metformin is absorbed from the lumen of the gastrointestinal tract (GI) through plasma membrane monoamine transporter (PMAT, or equilibrative nucleo-side transporter-ENT-4) (18). By its passage through the organic cation transporter 1 (OCT1), located in the basolateral membrane of human hepatocytes, metformin decreases hepatic glucose synthesis (19). Indeed, this was confirmed by investigations on OCT1 gene-deficient mice, where the uptake of metformin in hepatic and intestinal tissues was lower, compared to control animals (19). These studies implied that OCT1 is pivotal for raising the intracellular concentration of metformin; and as a corollary, there was a corresponding derangement in glucose metabolism (19). Interestingly, metformin is excreted unmetabolized through mutli-drug and toxin extrusion 1 (MATE1) and MATE2, located in the apical membrane of kidney proximal tubular cells, into urine (20). Recent studies suggest that substantial inter-individual heterogeneity in metformin pharmacokinetics exists, and this is recognized to be due to genetic variants of different metformin transporter proteins (20-22). Reduced expression or altered functionality of transporter proteins will result in less than optimum pharmacotherapy or undesirable toxic effects of metformin.
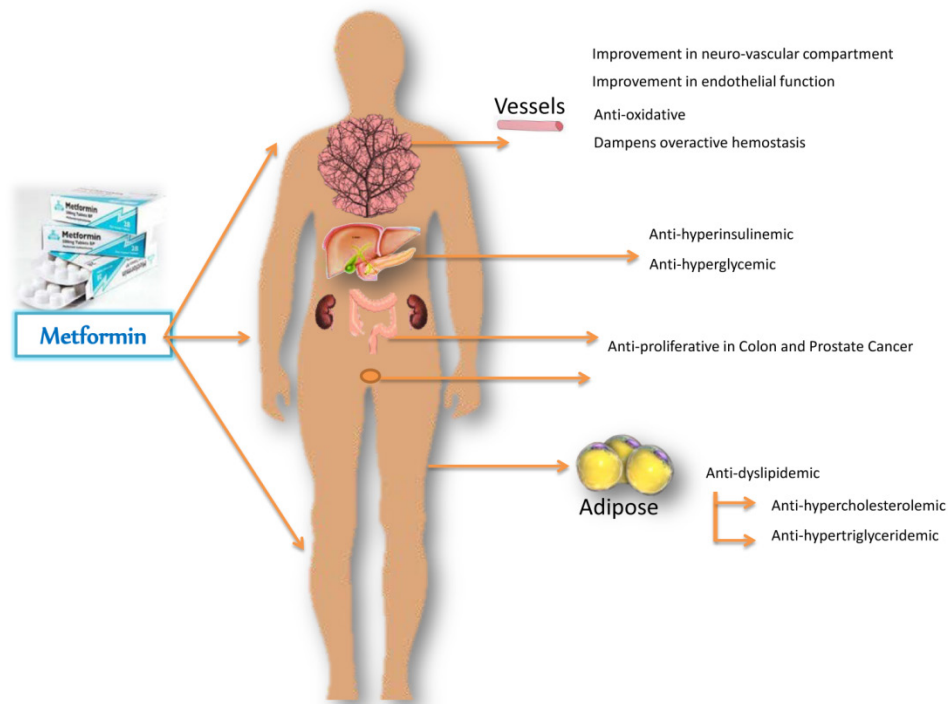

Figure 1. Metformin-mediated amelioration in diabetic and cancerous deranged metabolic profile, improvements in hemostasis and endothelial function, with regression of proliferative state. Metformin acts primarily on the liver and reduces glucose output, and secondarily on the peripheral tissues to increase glucose uptake. By decreasing gluconeogenesis, it ameliorates hyperglycemia in type 2 diabetes, improves endothelial function, oxidative stress, insulin resistance and fat redistribution. Accumulating evidence supports the antiproliferative role of metformin in colon and prostate cancer.
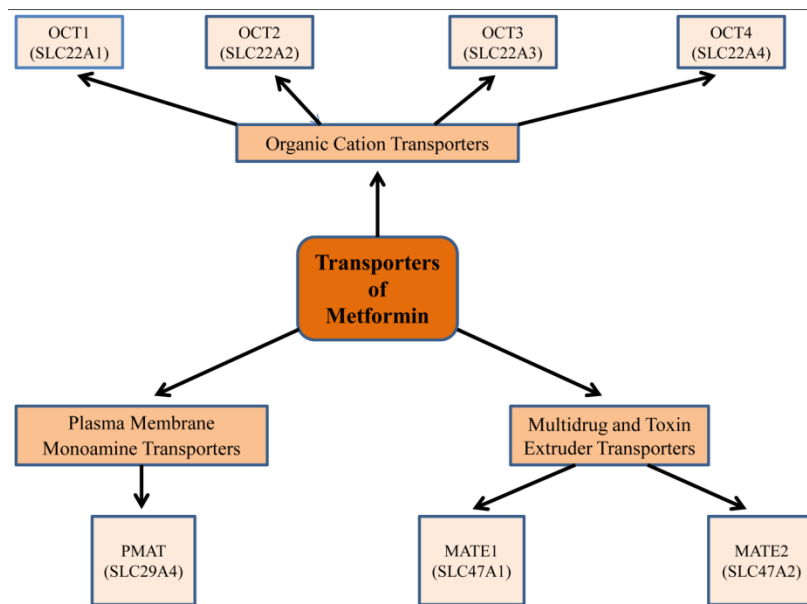
Figure 2. Metformin transporters: Isoforms and genes that demonstrate a role in metformin pharmacokinetics, pharmacogenetics, and thus have an impact on its pharmacological efficacy. Metformin is absorbed from the lumen of the gastrointestinal tract through plasma membrane monoamine transporter (PMAT). It requires the organic cation transporters (OCTs), located in the basolateral membrane of human hepatocytes, to be transported into the liver, thus decreasing hepatic glucose synthesis. The multidrug and toxin extrusion 1 and 2 (MATE1 and MATE2), located in the apical membrane of kidney proximal tubular cells, facilitate metformin excretion into urine. Genetic variation in transporter genes may alter transporter expression and functionality and thus metformin response.

Due to the reduced uptake of glucose from the intestinal tract, metformin improves insulin sensitivity by increasing peripheral glucose absorption and utilization by adipose tissue and skeletal muscle. It reduces hyperinsulinemia and improves insulin resistance by enhancing the affinity of insulin receptor for insulin (23). Moreover, metformin-driven benefits negate dyslipidemia by creating a milieu to give rise to lower circulating levels of total cholesterol, low-density lipoprotein (LDL) and triglycerides (24). In addition, administration of metformin to patients promotes lower body weight or at least weight neutrality (25, 26). Importantly, metformin is a low cost drug with a well characterized safety profile in management of diabetes and cancer.

3. Pleiotropic effects of metformin

Multifactorial mechanisms account for metformin's therapeutic contribution to its anti-oncogenic properties. Metformin inhibits complex 1 of mitochondrial electron transport chain (27-29), and thereby attenuates oxidative respiration resulting in ATP/AMP ratio imbalance, which in turn activates liver kinase B1 (LKB1) and AMPK (29, 30). Dephosphorylation by protein phosphatase 2A (PP2A) reversibly decreases enzyme efficiency (inactivation) of AMPK. Interestingly, metformin interplay with cellular metabolic homeostasis extends to inhibition of AMP deaminase to increase the pool of AMP available for activation of AMPK (31). Thus, AMPK can choreograph a network of diverse molecular sig-

naling routes depending on the contextual requirements for physiological cellular homeostasis and/or pathophysiological states (Fig. 3).

4. Regulation of Energy Homeostasis

Metformin acts cooperatively with the upstream kinase LKB1 to activate AMPK, the master regulator of metabolic homeostasis, resulting in the attenuation of plasma concentrations of risk factors, namely glucose, insulin, triglycerides and cholesterol (9, 32, 33). Metformin, via AMPK, controls the activity and expression of key regulatory lipid enzymes that have been identified in metabolic reprogramming of cancers. Phosphorylated AMPK, by upstream metformin-mediated effect, markedly reduces the expression (mRNA and protein) of lipogenic transcription factor sterol regulatory element-binding protein-1 (SREBP-1) and hence its targeted genes are also downregulated such as fatty acid synthase (FAS) (30, 34), and 3-hydroxy-3-methyl glutaryl-CoA reductase (HMGR, membrane-bound enzyme), which is known to reduce HMG-CoA to mevalonate (35). In addition, AMPK directly inactivates HMGR by phosphorylation at serine 872, thus essentially abating cholesterol synthesis (36). Also, statins, which are reportedly used for anti-cancer therapy, lower cholesterol by inhibiting HMGR (37-39), which is up-regulated in many forms of cancers (40). Moreover, the activated form of AMPK alters the activity of another type of lipogenic enzyme, acetyl-CoA carboxylase (ACC), which exists in two isoforms: ACC1 (ACC-α) and ACC2 (ACC-β).

Phosphorylation of AAC1 switches on fatty acid synthesis through its metabolite malonyl-CoA, while ACC2 controls fatty acid oxidation (41). Contextually of interest is the silencing by small interfering RNA (siRNA) of ACC1 gene that leads to growth arrest and caspase-dependent apoptosis of the human lipogenic LNCaP prostate cancer cell line (42). Altogether, these inhibitory signaling arrays of metabolic activity mitigate the burden of cancer-promoting effect of high fat diets.

5. Cell cycle arrest and anti-proliferative activity

Cellular growth-retarding contributions of metformin occur downstream from activation of LKB1/AMPK pathway, inhibition of mTOR, stabilization of the transcription factor p53 (its degradation is suppressed with downregulation of cyclin D1 (43) and associated increases in the expression of cyclin-dependent kinase inhibitors p27[Kip1] and p21[Cip1]

(44). The transcription factor p53 contributes to p21 gene transcription; and up-regulates the expression of apoptotic genes (Bax, Caspase-3, 8, 9) (45). These molecular signaling events channel cells to latency and hence drive them to accumulate in the G_o/G_1 cell cycle phase; DNA-damage and fragmentation ensues, leading to tumor suppression and further to autophagy and/or apoptosis (46). In addition, metformin-activated AMPK phosphorylates ULK1 at serines 317 and 777 to ultimately induce mitophagy, autophagy as well as cell death, and subsequently apoptosis and reduction of tumor size (47, 48). Furthermore, NF-κB is a transcription factor associated with apoptosis, inflammation, oxidative stress and neoplastic malignancy, which are all inhibited by metformin-stimulated AMPK (48-51). Evidently all of this is reflected by the aforementioned investigations on cell lines, animal models and beneficial survival outcomes of therapeutic intervention studies with metformin for cancer-related patients with T2DM.
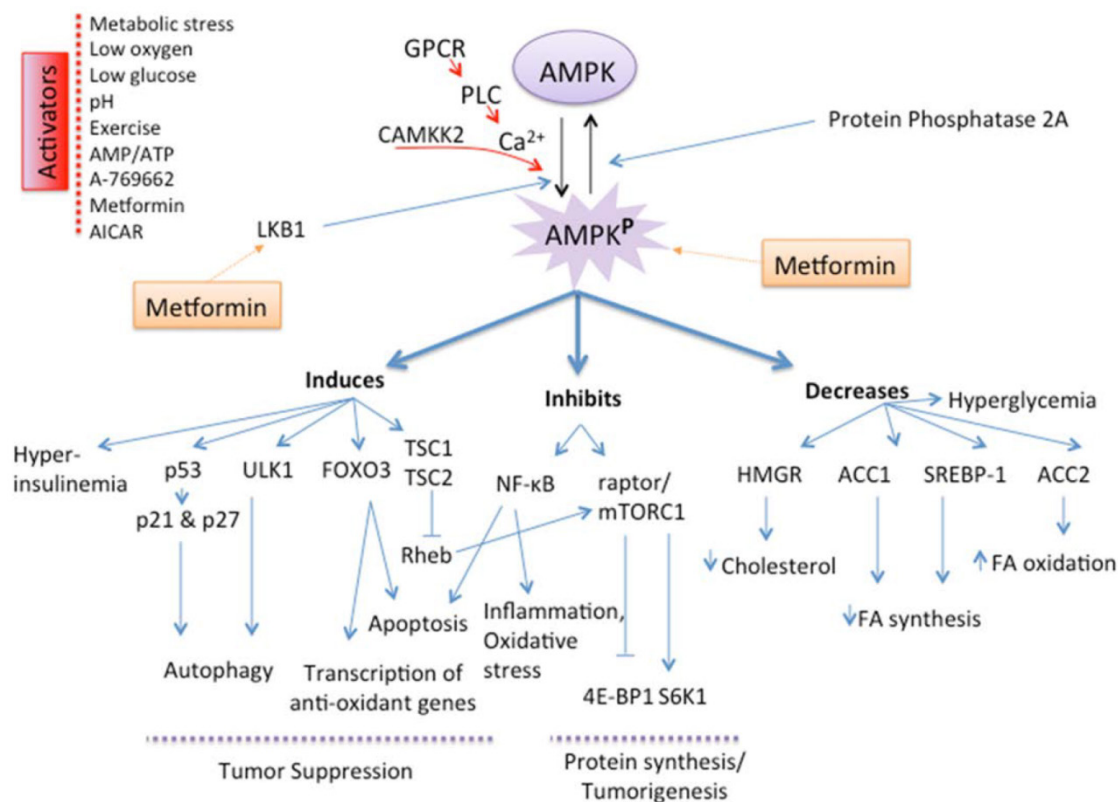

Figure 3. Mechanism and Role of AMPK activation. AMP-activated protein kinase (AMPK), a serine/threonine kinase, is an energy sensor whose activity is regulated by glucose. AMPK activation, secondary to a change in the AMP/ATP ratio, activation by upstream kinases, such as CAMKK (CaMK kinase) and LKB1, or administration of metformin by direct activation of LKB1, slows metabolic reactions that consume ATP and stimulates reactions that produce ATP, thereby restoring the AMP/ATP ratio and the normal cellular energy stores. AMPK activation will in turn induce catabolic pathways, such as fatty acid oxidation by inactivating acetyl CoA carboxylase (ACC2), and will inhibit anabolic pathways, such as fatty acid synthesis, mediated by ACC1. The mTOR pathway suppresses apoptosis via its effect on the tumor suppressors p53 and p27 and inhibits autophagy by suppressing UNC-51-like kinase 1 (ULK1) and ULK2. AMPK activation downregulates the tumorigenic effects of mTOR through the TSC1/TSC2 complex, thus leading to increased apoptosis and autophagy-mediated cell death. AMPK activation also inactivates P70S6K and 4E-BP1 subsequently inhibiting protein synthesis. AMPK activation regulates the transcription factor FOXO3, which in turn increases antioxidant gene expression.

Antiproliferative actions of AMPK, activated by metformin, suppress the growth of human umbilical vein endothelial cells (HUVEC) (52) as well as human and rat aortic smooth muscle cells (16, 53), thus preventing tumor angiogenesis (54). Intuitively, this will lead to starving of the tumor of nutrients, thereby limiting biosynthetic processes for macromolecules and hence cell growth for malignancy. Therefore, such multi-actions of AMPK suppress tumors with greater efficacy. Also, AMPK directly phosphorylates TSC2 tumor suppressor (phosphorylated at dual locations: threonine 1227 and serine 1345 (55) and raptor (regulatory-associated protein of mTOR, phosphorylated at serines 722 and 792) (56), a constituent subunit of mTORC1 (mammalian target of rapamycin), to suppress mTORC1 activity (55, 56). Moreover, metformin directly interacts with mTORC1 to inhibit its effects downstream, the ribosomal protein S6, S6 kinase 1 (S6K1) and the translation initiation factor 4E binding protein 1 (4E-BP1), and again this induces cell cycle arrest and inhibits DNA synthesis (57).

6. Regulation of oxidative stress

Metformin arrests hyperinsulinemia-induced free radicals (superoxide anion radical, hydroxyl radicals) and reactive oxygen species (ROS, hydrogen peroxide), which are integral to oxidative stress (imbalance between oxidants and antioxidants) and play an active mechanistic role in most cancers, and cell death is associated with generation of ROS (50, 58-61). AMPK in conjunction with the transcription factor FOXO3 lessens the fatty acid-induced generation of intracellular ROS by up-regulating the expression of the antioxidant thioredoxin (62-64), and the reduction in NADPH oxidase activity (65). In contrast, reactive oxygen species, such as hydrogen peroxide, significantly activates the AMPKα sub-unit to inhibit mTORC1 signaling and induce apoptosis (66).

7. Evidence from animal models and clinical trials

Taken together, this implies that metformin may lower cancer risk owing to its inherent properties of improving an altered metabolic picture, which is reflected by a fall in plasma glucose and insulin concentrations (32). Evidence from a panel of cancer cell lines (43, 67, 68), animal models (43, 68, 69) and epidemiological studies (70-73) suggests that metformin not only improves the life style and well-being, but lowers the rates of mortality in CRC (74) and PC (75). Multi-ethnic epidemiological studies have revealed disparity between ethnic groups of patients with PC risk due to diabetes and/or different medication (metformin) (6, 73, 75). However, in one of the latter meta-analysis studies on PC, the authors added a ca-

veat that additional long-term investigations on the use of metformin for PC must be forthcoming for concrete confirmation of the available data (75). Appropriately, triggered by these promising anti-tumorigenic benefits of metformin, epidemiologists have initiated clinical trials centered on a variety of tumors, including colorectal (5 ongoing clinical trials) and prostate (3 ongoing clinical trials) cancers with diabetes, and these can be located at http://www.clinicaltrials.gov/ website.

Continuing from above, recent evidence has commenced a debate on whether AMPK is a suppressor of cancer or an oncogenic member of signaling cascade (76, 77). This is perhaps an issue of the extent of AMPK activation, where at low activity it may not be a potent anti-cancer agent, but sustained high activation may be critical for inhibition on growth and survival of tumors. In the same context, it is not clear from the literature if any dose response curves for metformin as a chemotherapeutic agent have been performed on AMPK activity. Consideration must be given to the subject of genetic polymorphism of organic cation transporters (OCT1 and OCT2), which may affect the population-based individual variability and hence clinical efficacy of metformin (78). Therefore, this necessitates further studies to ensure and to consolidate that the thesis on metformin-activated AMPK is water-tight as an inhibitor of proliferative growth in cancers.

To conclude, recent advances in pinpointing the molecular control points that orchestrate the myriad of transduction pathways is pivotal to personalized therapy for cancer and other treatments, and the metformin research has rightly focused on AMPK, the master metabolic sensor. On a cautionary note, tumors are heterogeneous by character, and as Mother Nature has its own surprises, therefore it may be prudent to target more than one molecule as this approach may be more efficacious. Overall though, the preceding statements confirm that metformin is as a mono-therapeutic agent with multi-fold targets (Table 1 and 2).

8. Perspectives

Although an arsenal of therapeutic drugs is available for the management of different categories of cancer with type 2 diabetes mellitus, metformin remains the most widely used medication for T2DM. Recently, AMPK has received considerable attention regarding its central role as a metabolic homeostasis sustaining rheo-transducer as well as an inter-link between signaling routes between diabetes and cancer. Further preclinical and clinical investigations are ongoing to evaluate the therapeutic benefits of metformin on AMPK activation as an anti-diabetic, an-

ti-proliferative, hypoinsulinemic, apoptotic and hence anti-carcinogenic agent. Indeed, results with metformin demonstrate that AMPK is an ideal target for activation in both CRC and PC, and moreover metformin has other beneficial pleotropic targets in addition to metabolic control. In this context, and considering the afore-mentioned paragraphs, its effects on aberrant homeostasis in T2DM, CRC and PC are truly remarkable. Hence, it potentially holds substantial promise as a positive dual modifier of deranged metabolic homeostasis and as an anti-neoplastic agent.

Table 1. From Bench to Bedside: the use of metformin from cultured cells to clinical trials.

Cell lines	Animal Studies	Clinical Trials		
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b). Ben Sahra I, Laurent K, Loubat A, Giorgetti-Peraldi S, Colosetti P, Auberger P, Tanti JF, Le Marchand-Brustel Y, Bost F, (2008). The antidiabetic drug metformin exerts an antitumoral effect in vitro and in vivo through a decrease of cyclin D1 level. Oncogene 27(25):3576–3586. Proliferation of DU145, PC-3, LNCaP cancer cells; xenografts of LNCaP, reduction of cyclin D1 protein level	b). Ben Sahra I, Laurent K, Loubat A, Giorgetti-Peraldi S, Colosetti P, Auberger P, Tanti JF, Le Marchand-Brustel Y, Bost F, (2008). The antidiabetic drug metformin exerts an antitumoral effect in vitro and in vivo through a decrease of cyclin D1 level. Oncogene 27(25):3576–3586.	Status	Study	Clinical trials.gov identifier
c). Ben Sahra I, Laurent K, Giuliano S, et al. Targeting cancer cell metabolism: the combination of metformin and 2-deoxyglucose induces p53-dependent apoptosis in prostate cancer cells. Cancer Research 2010;70:2465–75.	c). Huang X, Wullschleger S, Shpiro N, McGuire VA, Sakamoto K, Woods YL, McBurnie W, Fleming S, Alessi DR. Important role of the LKB1-AMPK pathway in suppressing tumorigenesis in PTEN-deficient mice. Biochem J. 2008 Jun 1;412(2):211-21.	Recruiting	Exercise and Metformin in Colorectal and breast cancer Survivors	NCT01340300
d). Zakikhani, M., Dowling, R.J., Sonenberg, N., Pollak, M.N., (2008). The effects of adiponectin and metformin on prostate and colon neoplasia involve activation of AMP-activated protein kinase. Cancer Prev Res (Phila Pa) 1: 369–375. Metformin-mediated AMPK activation decrease cell growth and protein synthesis	d). Tomimoto A, Endo H, Sugiyama M, Fujisawa T, Hosono K, Takahashi H, Nakajima N, Nagashima Y, Wada K, Nakagama H et al. 2008 Metformin suppresses intestinal polyp growth in ApcMin/C mice. Cancer Science 99 2136–2141.	Recruiting	Impact of pretreatment with Metformin on colorectal cancer Stem cells (CCSC) and related Pharmacodynamics markers	NCT01440127
	e). Algire C, Amrein L, Zakikhani M, Panasci L, Pollak M. Metformin blocks the stimulative effect of a high-energy diet on colon carcinoma growth in vivo and is associated with reduced expression of fatty acid synthase. Endocr Relat Cancer. 2010 Jun 1;17(2):351-60.	Recruiting	An open-labeled pilot study of biomarker response following short-term exposure to metformin	NCT01816659
	f). Hosono K, Endo H, Takahashi H, Sugiyama M, Uchiyama T, Suzuki K, Nozaki Y, Yoneda K, Fujita K, Yoneda M, Inamori M, Tomatsu A, Chihara T, Shimpo K, Nakagama H, Nakajima A, 2010. Metformin suppresses azoxymethane-induced colorectal aberrant crypt foci by activating AMP-activated protein kinase. Mol Carcinog. 49:662-671.	Not yet recruiting	The chemopreventive effect of Metformin in patients with familial adenomatous polyposis: double blinded randomized controlled study	NCT01725490
		ii). Search identifier at clinicaltrial.gov/ Metformin/prostate cancer/diabetes		
		Status	Study	Clinical trials.gov identifier
		Recruiting	Metformin-Docetaxel Association Colorectal and breast cancer Survivors	NCT01796028
		Active not recruiting	Metformin in castration - resistant Metformin on colorectal cancer Stem cells (CCSC) and related Pharmacodynamics markers	NCT01215032
		Recruiting	Castration compared to castration Plus metformin as first line treatment For patients with advanced prostate cancer	NCT01620593

Table 2: Epidemiological Investigations.

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g) Wright, J.L., Stanford, J.L., 2009. Metformin use and prostate cancer in Caucasian men: results from a population-based case-control study. Cancer Causes Control 20: 1617-1622.
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Competing Interests

The authors have declared that no competing interest exists.

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