The influence of metformin in the etiology of selected cancers

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Obesity, hypertension and glucose tolerance disorders have become a growing concern in recent years. It is estimated that over 220 million people suffer from diabetes. It is a condition conducive to cardiovascular diseases, nephropathy, retinopathy and neuropathy but also to the development of many types of cancer. Insulin resistance and hyperinsulinemia lead to increased concentration of insulin-like growth factors, activation of IGF-R receptors, activation of PI3K and Ras-Raf pathways and result in increased cell division. The greatest risk is associated with developing stomach, pancreatic, colorectal, esophageal and lung cancer as well as breast and cervical cancer. Numerous cohort studies have confirmed that diabetic patients treated with metformin show a lower cancer morbidity and mortality rate. The dominant mechanism of action is activation of the AMP-activated protein kinase (AMPK) pathway and inhibition of mTOR protein, the key protein to regulate cell growth, apoptosis, proliferation and protein synthesis. Many clinical trials are currently under way to investigate the effectiveness of metformin in the prevention and treatment of neoplasms.

Key words: metformin, oncological treatment, diabetes, AMPK, mTOR.

Introduction

According to data gathered by World Health Organization (WHO) from 2008, approximately 1.5 billion people aged 20 and older were overweight [BMI (body mass index) 25–29.9 kg/m^2]; more than 200 million men and almost 300 million women were classified as obese (BMI > 30 kg/m^2). Between 1980 and 2008 the number of obese people doubled. It is widely known that factors such as obesity, lack of physical activity, and high-carbohydrate diet are conducive to glucose tolerance disorder. According to WHO figures from 2011, over 220 million people are suffering from diabetes in the world. The International Diabetes Federation (IDF) estimates that by 2025 the number of people with impaired glucose tolerance will increase to 472 million (9% of the adult population), of whom around 40–50% will develop type 2 diabetes. Recent studies show that diabetes is conducive not only to cardiovascular diseases, nephropathy, retinopathy and neuropathy, but also to the development of many types of cancers [1–4]. Recent data have shown that metformin, a drug commonly used in diabetic patients, can significantly decrease the incident rate and death risk associated with malignant cancers. In this paper we would like to present the mechanism of metformin's anticancer action and its role in selected malignancies.

Diabetes and cancer

Despite a tremendous body of research conducted in this field, there are still many aspects of the pathomechanism associating diabetes with cancer development that remain unclear.

Intense protein synthesis and rapid cell division within cancerous tissue demand high energy consumption. Therefore the proliferating cells need to ensure themselves a constant delivery of the basic energy source that is glucose. In the case of hypoglycemia cancer cells rely on glycosylation to produce ATP (the Warburg effect) [5]. Some authors have shown that hyperglycemia is connected with increased risk of cancer. Park *et al.* [4] compared populations of patients with a fasting serum glucose level under 109 mg/dl and above 126 mg/dl and showed that the cancer-related mortality rate was 38% higher in the latter group. The highest mortality in that group was associated with gastric cancer (HR: 1.52; 95% CI: 1.25–1.84) and lung cancer (HR: 1.48; 95% CI: 1.18–1.87). Jee *et al.* came to similar conclusions [6]. In this Korean study a significant increase in mortality rate was found among people with the fasting serum glucose level over 140 mg/dl when compared with a group with the level under 90 mg/dl (HR: 1.29; 95% CI: 1.22–1.37 among men and HR: 1.23; 95% CI: 1.09–1.39 among women). In the male population the highest death rate was associated with esophageal (HR: 1.44; 95% CI: 1.08–1.93), liver (HR: 1.57; 95% CI: 1.40–1.76), pancreatic (HR: 1.91; 95% CI: 1.52–2.41) and colorectal (HR: 1.31; 95% CI: 1.03–1.67) cancer. The highest death

rate among women suffering from diabetes with fasting blood glucose level in the morning over 140 mg/dl was associated with liver (HR: 1.33; CI: 1.01–1.81), lung (HR: 1.50; CI: 0.94–1.78), breast (HR: 1.24; CI: 0.65–1.92) and cervical (HR: 1.81; CI: 1.03–3.15) cancer; however, no such association was found for gastric and colorectal cancer.

On the other hand, in most cases normoglycemia allows for a concentration of glucose appropriate to meet the needs of cancerous cells [7]. A meta-analysis by Johnson and Bowker [8] assessed the benefits of an intensified glycemic control in patients with type 2 diabetes in the context of development of neoplasms. In the conclusion of the large randomized study which included populations of many thousands of patients, the authors did not find any decrease in the risk of cancer mortality rates among patients with type 2 diabetes with normal blood glucose level and HbA_{1c}. These results do not support the thesis that hyperglycemia is linked to increased carcinogenesis.

In an experimental model for the study of the influence of glucose concentration on tumor growth, Tannock and Kopelyan [9] found that higher glucose concentration increased the rate of cell division. However, at a glucose level of 5 mmol/L the intensity of cell division plateaus and any further increase in concentration does not lead to an increased rate of cell division. This suggests that hyperglycemia has no effect on cell proliferation.

Therefore, hyperinsulinemia rather than hyperglycemia seems to be the factor inducing intense cell proliferation. Activation of insulin receptors and insulin-like growth factor receptors (IGF-R) localized on the surface of cancer cells leads to cell proliferation [10]. Insulin resistance and hyperinsulinemia lead to increased concentration of insulin-like growth factors, activation of IGF-R receptors and activation of PI3K and Ras-Raf pathways, and result in increased cell division.

Metformin – anticancer drug?

Metformin has been successfully used for many years to treat patients with type 2 diabetes. A multicentre, randomized study conducted by UKPDS (United Kingdom Prospective Diabetes Study) [11] in patients with type 2 diabetes showed that treatment with metformin reduced the risk of myocardial infarction and diabetes-related death by 30% as compared with treatment with insulin and sulphonylurea derivatives. Numerous cohort studies have confirmed the thesis that patients with diabetes treated with metformin also have a lower risk of cancer incidence and mortality [12, 13].

Evans et al. [12] found a 23% reduction in cancer incidence among patients treated with metformin as compared with sulphonylurea derivatives. Another Canadian study [14] during a five-year follow-up of 10 309 people with diagnosed type 2 diabetes showed a significant cancer-related mortality reduction among patients receiving metformin in comparison to patients treated with oral sulphonylurea derivatives or insulin (3.5% vs. 4.9% vs. 5.8%). A Scottish cohort study [13] conducted among 12 255 people suffering from type 2 diabetes showed a 7.3% rate of newly diagnosed cancer among patients treated with metformin as compared with

11.6% of patients treated with other drugs. The median time between the diagnoses of diabetes and cancer was 3.5 and 2.6 years respectively (p = 0.001). Decensi et al. [15] found in turn in their meta-analysis a 31% reduction of cancer incidence and mortality in diabetic patients taking metformin.

Metformin belongs to the biguanide class derived from goat's rue (Galega officinalis), a herbaceous plant used in Ancient Egypt and medieval Europe [16–18]. It was first synthesized in 1922 by two scientists, Werner and Bellow from Trinity College, Dublin. The first biguanides (metformin and phenformin) were introduced in France in the 1950s after numerous publications by Jeane Sterne. They were withdrawn from the market after several years due to their severe adverse effects (phenformin) with particular focus on an increased risk of lactic acidosis. Having scored highly in many drug safety clinical trials, metformin (1,1-dimethylbiguanide) was introduced to the U.S. market in 1995 and gained more popularity with every year. The most common adverse effects after regular use of this drug are associated with the gastrointestinal tract, e.g. nausea, flatulence, vomiting, diarrhea. A small percentage of patients experience hives or other skin lesions. Lactic acidosis is very uncommon and affects mostly elderly people who suffer from circulation, liver or kidney failure. Apart from diabetics, metformin has also been successfully used in women with polycystic ovary syndrome, increasing the ovulation rate, and improving glucose tolerance and disordered lipid balance [19–21].

The hypoglycemic effect of metformin relies on the direct inhibition of gluconeogenesis and glycogenolysis in the liver. Metformin increases anaerobic glycolysis of peripheral tissue, particularly in skeletal muscles. Furthermore, it has an ability to enhance the number of GLUT4 glucose transporters and insulin receptors.

Metformin's mechanism of action is complex and may involve several pathways. The processes influenced by this drug include the following:
• activation of LKB1 (liver kinase B1)/AMPK (AMP-activated protein kinase) pathway,
• inhibition of cell division and/or promotion of apoptosis,
• promotion of autophagy,
• down-regulation of circulating insulin,
• activation of the immune system.

It is recognized that the basic mechanism of metformin action involves AMP-activated serine/threonine kinase, a type of protein that plays a key role in the regulation of cellular metabolism of both normal and cancer cells. AMP-activated protein kinase becomes activated under conditions of cellular energy deficit and a low AMP:ATP ratio. This resets the metabolic pathways from anabolic toward catabolic processes [22] and to promotion of e.g. glycolysis and oxidation of fatty acids; at the same time pathways of gluconeogenesis, protein, cholesterol and fatty acid synthesis are stopped [23]. AMP-activated protein kinase activation by metformin is mediated by LKB1 [24]. Liver kinase B1 is a suppressor protein. Its mutation is found in the hereditary Peutz-Jeghers syndrome characterized by the development of many hamartomatous polyps, skin lesions in the form of lentigines and a tendency to develop multiple carcinomas, particularly of the small intestine, stomach, pancreas and colon.

The activation of AMPK by LKB1 leads to phosphorylation of tuberous sclerosis complex 2 (TSC-2), which in turn inhibits the mTOR protein (mammalian target of rapamycin), a key protein which regulates processes of cell growth and angiogenesis, and promotes cell division and protein synthesis. The inhibiting effect of AMPK on mTOR results in blocking of the PI3K/PKB/Akt pathway, thus downregulating the synthesis of many proteins, e.g. eukaryotic initiation factor 4e-binding protein-4E-SPs, ribosomal protein S6 kinase, responsible for mitotic promotion [25, 26] (Fig. 1). Metformin may also inhibit mTOR dependent pathways in the absence of AMPK, LKB1 and TSC2 in a Rag GTPase dependent manner [27]. AMP-activated protein kinase also promotes cellular autophagy through the phosphorylation of cyclin-dependent kinase inhibitor protein, p27 [28].

The role of metformin in oncology

As mentioned, metformin significantly lowers the death risk associated with malignant cancers. If we look closer at the figures from the numerous studies [15, 29, 30] we will see that this phenomenon applies only to some types of cancer, e.g. esophageal, liver, colorectal, pancreas, breast and lung. In a Taiwanese study, Lee *et al.* [29] found a substantial reduction in liver and colorectal cancer morbidity among patients taking metformin (HR: 0.58 and 0.38, respectively).

Breast cancer

Obesity and diabetes are acknowledged risk factors of breast cancer for peri- and postmenopausal women. According to Thor and Anderson [31] the risk increases by 20% and the cancers very often are estrogen receptor positive.

Many studies conducted on breast cancer lines have shown positive action of metformin on these malignant cells. Alimova *et al.* [33] studied in diverse subtypes of breast cancer cell lines the effect of metformin on receptor expression and molecular pathways that participate in cell proliferation and apoptosis. Metformin inhibited cell proliferation by blocking the cell cycle at the G1 checkpoint, and downregulated cyclin D1 and transcription factor E2F1 expression, with no impact on other cell cycle regulators, such as p27kip1 or p21WAF1. It has been proven that metformin inhibits the MAPK (mitogen activated protein kinase) signaling pathway as well as Act activity [34]. Another mode of metformin activity is suppressing the phosphorylation of signal transducer and activator of transcription Stat3, also responsible for cellular proliferation and apoptosis, angiogenesis and activation of expression of genes related to breast cancer survival [31]. The cytotoxic effect of metformin on breast cancer cells may also take place via damage to the poly(ADP-ribose) polymerases (PARPs) – enzymes responsible for genome integrity – and via activation of caspase associated with cell apoptosis [31, 35]. High and low-concentration metformin in *in vitro* studies inhibited erbB2 expression and showed biological activity against estrogen receptors (ER) [30]. According to Liu *et al.* [36] metformin *in vitro* and *in vivo* (nude mice strain) exerts an inhibiting effect
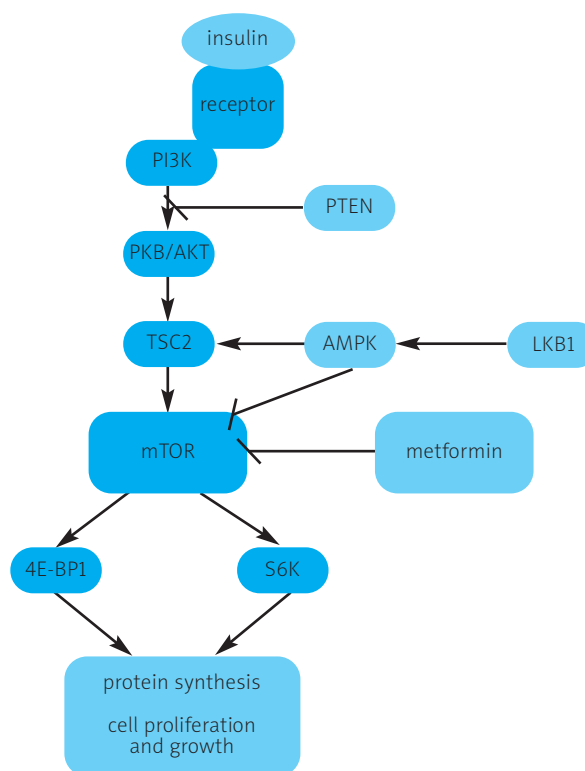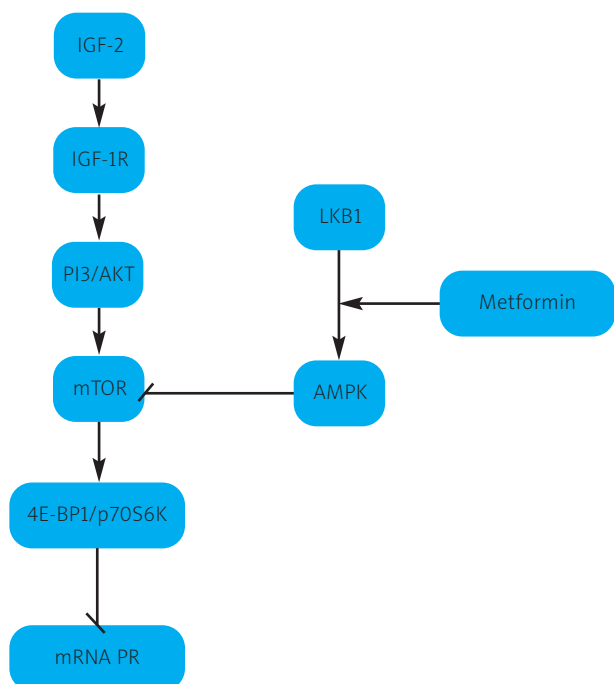

PTEN – phosphatase and tensin homologue deleted on chromosome 10; PI3K – phosphatidylinositol-3-kinase; PKB/AKT – protein kinase B/Akt; mTOR – mammalian target of rapamycin; TSC-2 – tuberous sclerosis complex 2; LKB1 – liver kinase B1; AMPK – AMP-activated protein kinase; 4E-BP1 – eukaryotic initiation factor 4E-binding protein; S6K – protein S6 kinases

Fig. 1. The role of metformin in the etiology of cancer. Adapted from: Cancer Res 2007; 67: 10804-10812 [25] – reprinted with permission

in cell culture conditions and reduces tumor mass in animal models. Bernstein *et al.* [37] tested receptor status in carcinoma cells after previous surgery in 90 female patients with diabetes, treated with metformin and other antidiabetic medication. In their work they noted that the frequency of progesterone-positive receptors in women treated with metformin was significantly higher in comparison to the group treated with other medication and these results may be used in breast cancer therapy.

Scientists from Texas [32] analyzed responses to neoadjuvant chemotherapy for early-stage breast cancer in 2529 women over a 17-year period – 2374 women were nondiabetic, 68 diabetic using metformin and 87 diabetic using other treatment – and reported a complete pathological remission in 24% of women taking metformin versus 8% of women taking other antidiabetic medication and 16% of nondiabetic patients ($p = 0.02$). More randomized studies are necessary to ascertain whether metformin improves responses to chemotherapy in women suffering from breast cancer and whether there is a benefit of adding this drug to conventional treatment [38, 39].

IGF-2 – insulin-like growth factor 2; IGR-1R – insulin-like growth factor receptor 1; PTEN – phosphatase and tensin homologue deleted on chromosome 10; PI3K/AKT – phosphatidylinositol-3-kinase/Akt; mTOR – mammalian target of rapamycin; AMPK – AMP-activated protein kinase; LKB1 – liver kinase B1; 4E-BP1 – eukaryotic initiation factor 4E-binding protein; p70S6K – 70 protein S6 kinases; mRNA PR – progesterone receptor mRNA

Fig. 2. The effect of metformin on PR expression. Adapted from: J Steroid Biochem Mol Biol 2011; 126: 113-120 [50] – reprinted with permission

Ongoing phase II and III clinical trials evaluate its role in breast cancer, where mTOR is suggested to be the shield [40].

During the recent ASCO (American Society of Clinical Oncology) meeting (2011) it was reported that the Clinical Trials Group from the National Cancer Institute of Canada has initiated enrollment of over 3500 women with node-positive and node-negative breast cancer who will receive metformin compared with placebo for 5 years [41].

Colorectal cancer

Colorectal cancer risk is higher in obese patients with type 2 diabetes. Patients treated with insulin are twice as likely to develop this type of cancer as compared to patients taking metformin [42]. It has been supported that aberrant crypt foci (crypts of Lieberkuhn) may play a role in colon cancer pathogenesis [43].

The inhibiting effect of metformin on intestinal epithelial cell proliferation and the development of aberrant crypt foci and polyps has been shown in an animal model. The mechanism of metformin-mediated suppression of the abovementioned precancerous states occurred via the most common way – through AMPK activation and mTOR inhibition [43].

Studies of colon cancer cell lines prove that AMPK activation is accompanied by lower expression of VEGF as well as GLUT1 and COX-2 glucose transporter, which may additionally result in cell apoptosis [46]. Other studies have shown that metformin also induces apoptosis in suppressor p53-deficient colon cancer cells [47].

The findings of these studies show that type 2 diabetic patients constitute a high risk group for colon cancer and suggest a preventive effect of metformin.

Pancreatic cancer

Jee *et al.* [6] estimated whether the increase in risk of death from pancreatic cancer depended on duration of diabetes. The scientists found that the mortality risk increased according to diabetes duration with a twofold rise in mortality for diabetes duration of less than 5 years and a threefold rise for diabetes duration of more than 10 years. The evidence provided by an American study [48] exploring the relationship between metformin and pancreatic cancer risk proved the protective effect of the drug. According to the authors it reduces the risk of pancreatic cancer by over a half (HR: 0.38, 95% CI: 0.22–0.69; $p = 0.001$).

Other cancers

In breast and endometrial cancer alike, the presence of progesterone receptors (PR) plays a vital role. A study by Cui *et al.* [49] established a significant IGF1-induced reduction in the number of PR in breast cancer cells. This phenomenon has been linked with the activity of the PI3K/Akt/mTOR pathway and inhibition of PR gene transcription. Similar results were obtained by Xiu *et al.* [50] in an examination of endometrial cancer cell lines. The results of their study show that IGF2 mediates activation of the PI3K/Akt/mTOR pathway, 4E-BP and p70S6K phosphorylation and a significant increase in cell proliferation, while both IGF1 and IGF2 inhibit expression of the PR A/B gene (Fig. 2). The authors also demonstrated that metformin exerts a counteracting effect on the abovementioned processes and this may imply that introduction of this drug combined with progesterone supplementation would result in a better response to treatment.

Ongoing clinical trials

The promising results of many cohort and experimental studies triggered further clinical trials which aim to determine whether this inexpensive, generally available and safe medication can reduce cancer incidence or improve cancer prognosis. Another interesting issue demanding further studies is the aspect of metformin use in nondiabetic patients. Could this group also benefit from metformin treatment? Currently many phase I, II and III trials are under way to study metformin's effect on breast, prostate, endometrial and pancreatic cancer (Table 1). Perhaps in the near future metformin's antineoplastic activity will be generally recognized and eventually it will be applied in oncotherapy.

Table 1. A selection of ongoing clinical trials determining metformin's effect on oncologic diseases

Type of cancer	Official title	Trial identifier no	Estimated enrollment	Purpose of the study
prostate cancer	Metformin in Castration-Resistant Prostate Cancer	NCT01215032	106	prospective study to evaluate metformin's effect on PSA concentration in prostate cancer patients receiving androgen deprivation therapy
pancreatic cancer	Combination Chemotherapy With or Without Metformin Hydrochloride in Treating Patients With Metastatic Pancreatic Cancer	NCT01167738	82	randomized phase II trial to evaluate the effectiveness of combination chemotherapy with or without metformin hydrochloride in treating patients with metastatic pancreatic cancer
endometrial cancer	Metformin and Endometrial Cancer	NCT01205672	30	non-randomized phase I trial to evaluate the effect of metformin on s6k expression in endometrial cancer
colorectal cancer	A Trial of Metformin for Colorectal Cancer Risk Reduction Among Patients With a History of Colorectal Adenomas and Elevated Body Mass Index	NCT01312467	39	a phase II study to evaluate the potential preventive effect of metformin in patients with history of colorectal adenomas and a BMI > 30
breast cancer	A Phase III Randomized Trial of Metformin vs Placebo in Early Stage Breast Cancer A Phase III Randomized Trial of Metformin vs Placebo in Early Stage Breast Cancer	NCT01101438	3582	a phase III study to evaluate the effect of the addition of metformin to standard chemotherapy in women with early-stage breast cancer.
breast cancer	Metformin Hydrochloride in Treating Women With Stage I or Stage II Breast Cancer That Can Be Removed By Surgery	NCT00984490	30	a phase II study to evaluate the effect of metformin on cell proliferation (Ki67) and apoptosis (kaspaza 3) in women with stage I or stage II cancer
breast cancer	Metformin Pre-Surgical Pilot Study	NCT00930579	35	a phase II study to evaluate the effect of metformin on the AMPK/mTOR pathway in women with newly diagnosed early-stage breast cancer.
breast cancer	The Impact of Obesity and Obesity Treatments on Breast Cancer	NCT00933309	24	phase I study to evaluate the effect of aromatase inhibitor treatment in combination with metformin and rosiglitazone in obese women with estrogen receptor positive breast cancer
breast cancer	Clinical and Biologic Effects of Metformin in Early Stage Breast Cancer	NCT00897884	40	to evaluate the effect of pre-operative metformin therapy on cell proliferation in tumor tissue in women diagnosed with breast cancer
breast cancer	A Trial of Standard Chemotherapy With Metformin (vs Placebo) in Women With Metastatic Breast Cancer	NCT01310231	78	randomized phase II trial to evaluate standard chemotherapy with metformin on PFS in women with metastatic breast cancer.
breast cancer	Effect of Metformin on Breast Cancer Metabolism	NCT01266486	40	to measure metformin induced effects in phosphorylation of S6K, 4E-BP-1 and AMPK
breast cancer	The Use of Metformin in Early Breast Cancer Patients Pre-Surgery	NCT01302002	30	to evaluate the effects of metformin on proliferation (Ki67) and apoptosis (TUNEL) in women with stage I or II breast cancer

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