Molecular Pathways

Clinical Cancer Research

Metformin: A Therapeutic Opportunity in Breast Cancer

Ana M. Gonzalez-Angulo[1,2] and Funda Meric-Bernstam[3]

Abstract

Two important, related pathways are involved in cancer growth: the insulin/insulin-like growth factor-1 (IGF1) signaling pathway, which is activated when nutrients are available, and the adenosine mono-phosphate-activated protein kinase (AMPK) pathway, activated when cells are starved for carbohydrates. Metformin inhibits transcription of key gluconeogenesis genes in the liver, increases glucose uptake in skeletal muscle, and decreases circulating insulin levels. Metformin reduces levels of circulating glucose, increases insulin sensitivity, and reduces insulin resistance-associated hyperinsulinemia. At the level of cell signaling, metformin activates AMPK. There are extensive preclinical data showing the anticancer effects of metformin in all breast cancer subtypes as well as in cytotoxic therapy-resistant models. These data, and the epidemiological and retrospective data supporting the antineoplastic effects of metformin, provide the rationale to study the role of metformin for breast cancer therapy in a variety of clinical settings. *Clin Cancer Res; 16(6); 1695–700. ©2010 AACR.*

Background

Energy signaling and cancer. Cell growth and proliferation are regulated coordinately by multiple signals, including growth factors, availability of nutrients, and energy (intracellular ATP). The insulin/insulin-like growth factor-1 (IGF1) signaling pathway is activated when nutrients are available, whereas the adenosine monophosphate-activated protein kinase (AMPK) pathway, a sensor of cellular energy, is activated when cells are starved for energy (1). In mammals, insulin promotes lipid, protein, and glycogen synthesis, whereas AMPK inhibits these biosynthetic pathways. The effect of insulin on protein synthesis is mediated in part by activation of the mammalian target of rapamycin (mTOR) pathway via phosphorylation of tuberous sclerosis complex 2 protein (TSC2, also known as tuberin), whereas activation of AMPK causes phosphorylation of different sites on TSC2 and inhibits mTOR (2, 3). Thus, TSC2 integrates insulin and energy signaling to control cell growth and survival. Further, there is direct crosstalk between insulin and energy signaling. In some tissues, such as cardiac muscle, insulin antagonizes activation of AMPK by activating Akt (4). This in turn phosphorylates AMPKα on Ser485/Ser491, which reduces AMPK Thr172 phosphorylation by LKB1, and the resulting activation of AMPK (5). However, in processes that regulate plasma glucose levels, the insulin and AMPK signaling pathways work in the same direction (6). Activation of AMPK plays a role on the ability of muscle contraction to stimulate glucose uptake, and to increase the insulin sensitivity of glucose uptake with exercise (7). Although unclear, the mechanism for this effect may be due to the ability of AMPK to inhibit the mTOR pathway, which is activated by insulin and exerts a feedback regulation on insulin signaling by downregulating IRS1 (8–10). In the liver and in adipocytes, insulin and AMPK repress the expression of enzymes of gluconeogenesis (11), and suppress the activation of hormone-sensitive lipase, and lipolysis (12, 13), respectively.

IGF signaling has an important role in normal cell growth, but it is also a known mediator of the malignant phenotype. IGF1 receptor ligand binding leads to autophosphorylation of tyrosines at its kinase domain. This induces the phosphorylation of tyrosines and serines to form binding sites for insulin receptor substrates (IRS) and Src and subsequent activation of signaling via the phosphatidylinositol-3-kinase (PI3K)/Akt/mTOR and RAS/RAF/mitogen-activated protein kinase (MAPK) pathways (14–16). Regulation of IGF1R occurs at multiple levels including ligand availability (17), and intracellularly through Src, phosphatases, integrins, and the RACK1 scaffolding protein (18). Downstream, IGF1R effectors mTOR complex1 (mTORC1) and S6 kinase participate in the feedback suppression of the PI3K/AKT signaling (15, 18, 19). Although IGF1R is not unique in driving tumor cell proliferation, it is necessary for oncogene-induced cellular transformation and mediates both the survival and the proliferation signaling required for cell growth, which enables transformed cells to form tumors, and to survive detachment (14, 20). Further, preclinical studies have shown that IGF1R overexpression may induce tumor formation and metastasis (21, 22).

Authors' Affiliations: Departments of [1]Breast Medical Oncology, [2]Systems Biology, and [3]Surgical Oncology, The University of Texas, M.D. Anderson Cancer Center, Houston, Texas

Corresponding Author: Ana M. Gonzalez-Angulo, Department of Breast Medical Oncology, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 1354, Houston, TX 77030. Phone: 713-792-2817; Fax: 713-794-4385; E-mail: agonzalez@mdanderson.org.

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As stated above, mTOR activity is in part regulated by cellular energy levels and nutrients as well as oxygen and growth factors (23). When mTOR is deregulated, it leads to increased cell growth and proliferation. Further, the signaling pathways that control its activity are often deregulated in human cancers. For instance, activating PIK3CA mutations or loss of expression of the tumor suppressor PTEN, both prevalent in breast cancer, can lead to uncontrolled mTOR activity (24, 25) and subsequent translation of mRNAs that encode for growth factors, apoptosis inhibitors, activators of the cell cycle, and angiogenic factors all of which contribute to tumor formation and growth (26). As a result, the PI3K/Akt/mTOR signaling pathway is a prime target for anticancer therapies (27), and inhibiting the energy pathway through AMPK activation and mTOR inhibition is a potential mechanism to prevent and decrease cancer growth.

Figure 1 summarizes the biological effects of metformin inside and outside the cancer cell.

Metformin Activity in Breast Cancer

Metformin is a biguanide, and a widely prescribed oral medication used as front-line therapy for type 2 diabetes. Population studies suggest that metformin decreases the incidence of cancer and cancer-related mortality in diabetic patients (28, 29). Clinical and epidemiologic evidence links hyperinsulinemia, insulin resistance, and diabetes to poor breast cancer outcomes (30). Further, insulin can promote tumorigenesis via a direct effect on epithelial tissues, or indirectly by affecting the levels of other modulators, such as insulin-like growth factors, sex hormones, and adipokines (31–35). Exciting preclinical studies have shown that metformin can inhibit the growth of cancer cells, including breast cancer *in vitro* and of tumors *in vivo* (36–40). More recently, a retrospective study of patients who received neoadjuvant chemotherapy for breast cancer showed that diabetic cancer patients receiving metformin during their neoadjuvant chemotherapy had a higher pathological complete response rate than diabetic patients not receiving metformin (24% versus 8%, $P = 0.007$; ref. 41).

The antineoplastic effects of metformin in breast cancer are supported by a biological rationale involving important factors associated with breast cancer prognosis. In the liver, metformin inhibits transcription of key gluconeogenesis genes and increases glucose uptake in skeletal muscle. It reduces levels of circulating glucose, increases insulin sensitivity, and reduces insulin resistance-associated hyperinsulinemia (42). At the level of cell signaling, several mechanisms of metformin action have been proposed; the most important one relates to the activation of AMPK (43). AMPK, the central cellular key energy sensor with a unique ability to directly sense cellular energy, places it in an ideal position to ensure that cell division, which is a highly energy-consuming process, only proceeds if cells have sufficient metabolic resources (1, 44). Once activated, it leads to the suppression of many of the metabolic processes that depend highly on sufficient cellular ATP supply (gluconeogenesis, protein and fatty acid synthesis, cholesterol biosynthesis) and that promote catabolic processes (glycolysis, fatty acid beta oxidation; ref. 45). Further, the AMPK pathway exerts two inhibitory effects on mTOR, via phosphorylation of TSC2 and regulatory associated protein of mTOR (raptor). AMPK is activated when ATP levels are lower, switching off the mTOR pathway over the positive effects of amino acids (46) or growth factors via phosphorylation of TSC2 by AMPK, which stimulates its Rheb-GAP activity. Metformin and its analogs also activate AMPK in the absence of TSC2 through raptor phosphorylation (2, 47). This effect seems to be a direct effect on mTOR kinase activity, possibly involving increased binding of 14-3-3 proteins and/or partial dissociation of proline-rich Akt substrate of 40 kDa (PRAS40; ref. 48).

Clinical-translational Advances

Preclinical studies. Initial experiments showed that metformin was capable of reducing proliferation in prostate, colon, and breast cancer cell lines through cell cycle inhibition shown by an important decrease of cyclin D1 protein level. Subsequently *in vivo* experiments using intraperitoneal or oral metformin in nude mice resulted in tumor growth inhibition up to 55% (39). To evaluate the effect of metformin on cell proliferation, investigators looked at the effect of this drug *in vitro* on a group of breast, ovarian, and prostate cancer cells lines. In MCF-7 human breast cancer cells, metformin acted as a growth inhibitor rather than an insulin sensitizer. Further, they found that exposure to a growth inhibitory concentration of drug by means of the AMPK pathway activation and mTOR inhibition can lead to decreased protein synthesis, blocking both growth and proliferation (37, 38). Subsequent experiments looking specifically at breast cancer cell lines by hormone receptor status confirmed that AMPK stimulation by metformin results in complete cell growth inhibition in estrogen receptor-positive cell lines, but partial inhibition in the estrogen receptor-negative cell lines. Interestingly, there was a significant increase in vascular endothelial growth factor in estrogen receptor-negative cell lines. Furthermore, in estrogen receptor-negative cell lines, orthotopic MDA-MB-435 xenograft models, metformin treatment lead to increased tumor growth, increased cancer cell viability, and angiogenesis (49). In contrast, in a more recent report, investigators found that nude mice bearing tumor xenografts of the triple receptor negative cell line MDA-MB-231 show significant reductions in tumor growth and cell proliferation when treated with metformin, as compared with controls (50).

Lastly, there are now data showing that metformin selectively kills breast cancer stem cells. Investigators took four genetically different types of breast cancer cells and added metformin to doxorubicin. The combination was able to kill both non-stem and cancer stem cells in culture, and showed reduced tumor mass and prolonged remission more than with either drug alone in a xenograft mouse model (51).

These extensive preclinical data showing the anticancer effects of metformin in all breast cancer subtypes as well as

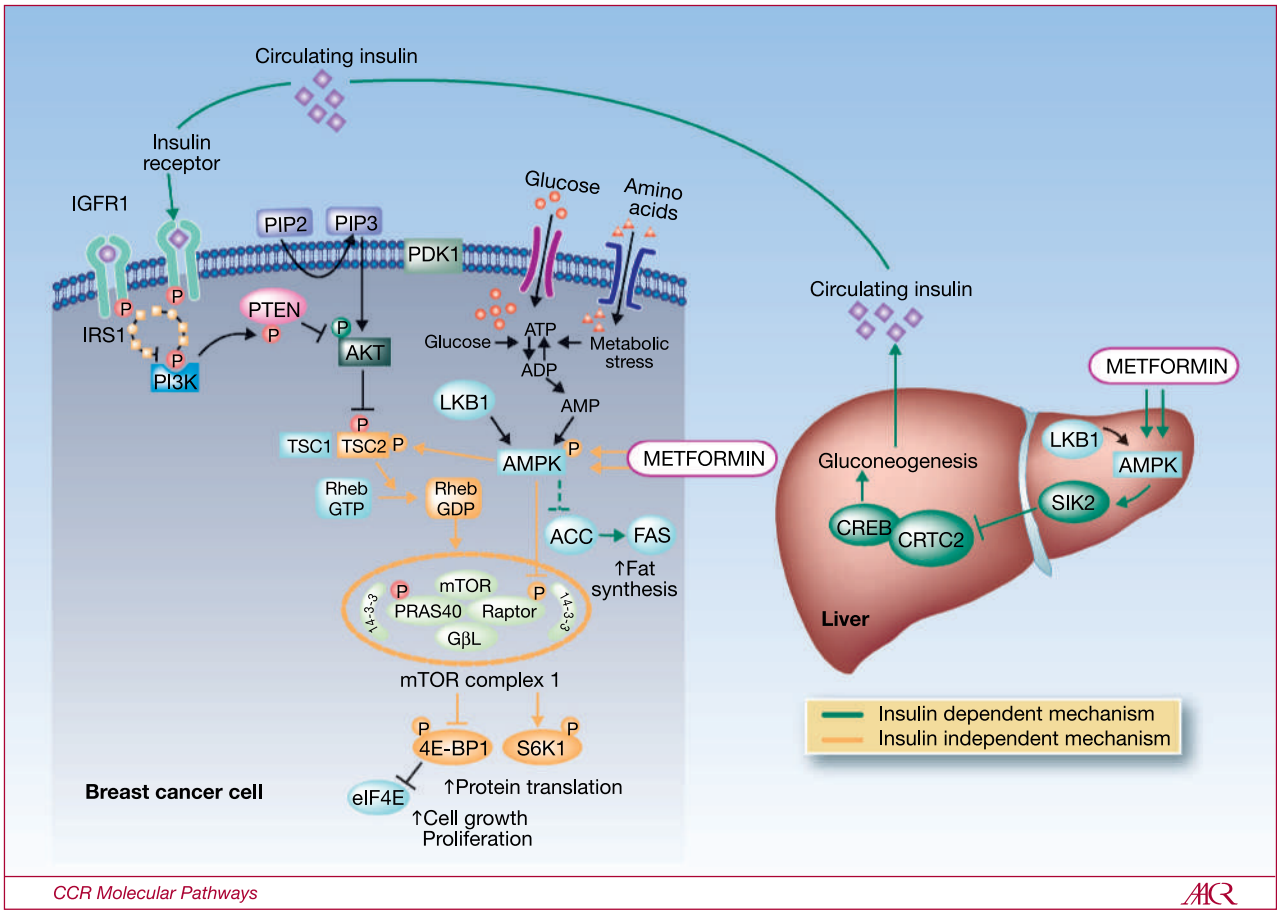

Fig. 1. Mechanisms of action of metformin. Metformin seems to exert its cell growth-inhibitory effects through two distinct mechanisms. A direct mechanism that inhibits the mTOR pathway and an indirect mechanism depending on insulin levels. Metformin activates AMPK, the cellular energy sensor. Activation of AMPK leads to suppression of many of the processes highly dependent on ATP, such as gluconeogenesis, protein, fatty acid, and cholesterol biosynthesis. It inhibits transcription of gluconeogenesis genes in the liver and increases glucose uptake in skeletal muscle. This seems to reduce the levels of circulating glucose, increase insulin sensitivity, and reduce the hyperinsulinemia associated with insulin resistance. In the cancer cell, the mTOR signaling pathway promotes cell growth and proliferation by interplay of two opposing upstream pathways involving the Akt pathway, which signals availability of nutrients, and the AMPK pathway, which signals lack of energy. TORC1 directly regulates cell growth and contains raptor and PRAS40, which represses mTOR activity. There are two types of input that activate TORC1: increased availability of amino acids at the cellular level and activated insulin or the related growth factor IGF1 signaling. They activate PI3K, which switches on Akt. TORC1 is stimulated by the active, GTP-bound form of the Rheb, and immediately upstream of Rheb is the TSC1:TSC2 heterodimer. TSC2 converts Rheb to its inactive Rheb:GDP form. Activation of Akt causes phosphorylation of TSC2, which is thought to inhibit its GAP activity and stimulates TORC1. On the other hand, Akt also phosphorylates PRAS40 and seems to relieve its inhibitory effect. AMPK is activated when ATP levels lower switching off the mTOR pathway over the positive effects of amino acids or growth factors via phosphorylation of TSC2 by AMPK, which stimulates its Rheb-GAP activity. Metformin and its analogs also activate AMPK in the absence of TSC2 through raptor phosphorylation. This effect seems to be a direct effect on mTOR kinase activity, possibly involving increased binding of 14-3-3 proteins and/or partial dissociation of PRAS40. So, the AMPK pathway exerts two inhibitory effects on mTOR via phosphorylation of TSC2 and raptor similarly to the Akt pathway, which exerts two stimulatory effects via phosphorylation of TSC2 and PRAS40.

in cytotoxic therapy-resistant models provided a rationale to study the drug in the clinic.

Clinical studies. The provocative results of metformin in retrospective clinical as well as in preclinical studies in breast cancer have lead to research in the clinic, both for treatment and prevention. Metformin is being investigated using different approaches.

Window of opportunity studies. In this setting, patients with early operable breast cancer of high risk breast epithelial neoplasia get a baseline biopsy, and then go on to receive either placebo or different doses of metformin for a short period of time (2-6 weeks); tissues are collected at the time of surgery. The main goal of this design is to evaluate whether the use of different doses of metformin can modulate tissue and serum biomarkers involved in cancer growth and to try to establish the lowest dose at which the drug modulation occurs. There are at least five on-going clinical trials world-wide looking at the molecular effects of metformin in breast cancer using this design (52).

Phase II, neoadjuvant randomized trials. In this setting, patients are randomized to receive a full course of

neoadjuvant systemic therapy with or without metformin. Tissues (tumor and blood) are collected from all patients at baseline, at a set time point during treatment and at the time of surgery. The purpose of these studies is to evaluate the benefit of combining metformin with the standard neoadjuvant systemic therapy. They look at clinical and pathological outcomes, but also look at the differences of tumor and blood biomarkers of pathway modulation and their correlation with the outcome endpoints. Although randomized, these studies may not be powered to definitively answer the clinical question, but may help set the basis for patient selection for subsequent confirmatory phase III studies. Several clinical trials are ongoing or planned targeting patients with hormone receptor-positive breast cancer and patients with HER2-positive breast cancer (53, 54).

Phase I-II trials in metastatic disease. These studies are usually done to find the dose limiting toxicities, safety, and initial efficacy data of the single agent or the combination with existing breast cancer therapies. Currently two of these trials are on-going. One trial focuses on obese patients with metastatic hormone receptor-positive breast cancer, and uses the combination of exemestane and avandamet (metformin and rosiglitazone), and the second one looks at the combination of metformin and temsirolimus, an mTOR inhibitor in solid tumors and lymphoma (52).

Phase III, Randomized, Placebo-Controlled Trials

These trials can be done in the therapeutic or in the prevention settings. In the therapeutic setting, the National Cancer Institute of Canada will conduct the MA-32 trial: a phase III randomized study of the effect of metformin versus placebo in early stage breast cancer. A total of 3,582 patients will be randomized to metformin 850 mg a day for 5 years versus placebo. Patients will be stratified by hormone receptor status, HER2 status, and chemotherapy use. The primary endpoint is invasive disease-free survival. A parallel large neoadjuvant phase III randomized trial in early breast cancer is being designed. Patients will be randomized to six cycles of docetaxel, doxorubicin and cyclophosphamide (TAC) plus or minus metformin. The primary endpoint will be pathological complete response. Tissue and blood collections for correlative studies are planned. At this time, there is insufficient data on the minimal dose needed to have an antiproliferative effect, as well as long-term safety of metformin in patients without diabetes. Thus further work is needed before initiation of phase III prevention studies.

Biomarkers

In general, an adequate predictive biomarker should be able to identify the tumors that are sensitive to a specific therapy. In order to develop such markers it is necessary to obtain serial biopsies to test a large range of pharmacodynamic endpoints and their correlation with clinical outcomes. The markers should be tested in independent sets, preferably in the setting of a randomized clinical trial. Currently there are several potential markers studied at the preclinical level that relate to metformin mechanisms of action. They constitute the ideal initial set to explore, and include components of the IGF1R axis, the AMPK and PI3K/Akt/mTOR signaling pathway, and metabolism serum markers such as insulin, c-peptide, and leptin. The above mentioned clinical trials should serve as the training and validation sets to develop biomarkers of response that can be used to personalize metformin-based cancer therapy.

Adverse Effects to Be Considered

Metformin has been extensively used in patients with type 2 diabetes and, less frequently, in patients with polycystic ovarian syndrome. The drug has shown a very good safety profile. The most common toxicity is gastrointestinal distress, including transient mild nausea and moderate diarrhea, with a few patients having to discontinue treatment because of the latter (54–56). The only potential major adverse event from metformin therapy is lactic acidosis. This condition, although rare, is limited to patients with renal and/or liver disorders, and it should be taken into consideration in cancer care, because these patients undergo diagnostic imaging tests requiring contrast media that can increase their risk for it. Rare side effects include hirsutism and vitamin B12 malabsorption after long-term exposure. No teratogenic effects have been reported (55).

Conclusion

Metformin is a widely prescribed oral medication used as front-line therapy for type 2 diabetes. It has been shown to inhibit the growth of cancer cell lines, including breast cancer, *in vitro* and *in vivo* tumor models. Population and retrospective studies showed that metformin decreases the incidence of cancer and cancer-related mortality, and increases the response to neoadjuvant chemotherapy in diabetic patients. Metformin induces AMPK activation, which decreases insulin levels and leads to inhibition of protein synthesis pathways, decreasing cancer cell proliferation and growth. As a result, metformin is being investigated as a therapeutic agent in different clinical settings for all breast cancer subtypes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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