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Metformin Activity against Breast Cancer: Mechanistic Differences by Molecular Subtype and Metabolic Conditions

Reema S. Wahdan-Alaswad and Ann D. Thor

Abstract

Obesity and type 2 diabetes increase the risk of and reduce survival in breast cancer (BC) patients. Metformin is the only anti-diabetic drug that alters this risk, with a reduction in BC incidence and improved outcomes. Metformin has AMP-kinase (AMPK) dependent and independent mechanisms of action, most notably affecting the liver and skeletal muscle. We and others have shown that metformin also downregulates protein and lipid synthesis; deactivates various receptor tyrosine kinases; alters cell cycle transcription/translation; modulates mitochondrial respiration and miRNA activation; targets key metabolic molecules; induces stem cell death and may induce apoptosis or autophagy in BC cells. Many of these anti-cancer effects are molecular subtype-specific. Metformin is most potent against triple negative (basal), followed by luminal BCs. The efficacy of metformin, as well as dose needed for the activity, is also modulated by the extracellular glucose concentration, cellular expression of the glucose transporter protein 1 (GLUT1), and the organic cation transporter protein 1 (OCT1, which transports metformin into cells). This chapter summarizes the diverse clinical and preclinical data related to the anti-cancer effects of metformin, focused against breast cancer.

Keywords: metformin, breast cancer, TGF- β , STAT3, and PI3K/AKT/mTOR, FASN, MiR-193b, cancer stem cells, EGFR, cholesterol, glucose

1. Introduction

Metabolic dysregulation of carbohydrate and lipid metabolism is frequent in cancer cells, facilitating growth and survival through adaptive mechanisms. Otto Warburg was the first to recognize that cancer cells favor glycolysis as compared to oxidative phosphorylation for the generation of energy (ATP) [1]. While the former is less efficient in terms of energy production per molecule of glucose, it also generates precursor molecules (amino acids, fatty acids, etc.) for replication and facilitates survival under oxidative stress [2]. This is in contrast to normal cells, which typically use oxidative metabolism to derive more energy (ATP) per molecule of glucose [3, 4]. Nearly a century later, we now recognize that cancer cells may utilize either aerobic or anaerobic respiration. The majority of cancer cells also have alterations of mitochondrial respiration, further providing a selective advantage to

facilitate cancer growth and survival [5]. More specifically, it may increase intracellular reactive oxygen species by disruption of the mitochondrial electron transport chain to reduce the mitochondrial membrane potential in BC or act directly to inhibit the mitochondrial respiratory-chain complex 1 (MRCC1) [6–8].

Chronic energy excess and physical inactivity lead to systemic alterations of carbohydrate and fatty acid metabolism characterized by systemic hyperglycemia, hyperinsulinemia with insulin resistance followed by hypoinsulinemia, an increase in inflammatory cytokines and adipokines, alterations of steroid and growth hormones, and downregulation of immune surveillance and tissue oxygenation [3, 9, 10]. These changes are frequent but variable in patients with obesity and type 2 diabetes and can be modified by drugs, exercise, body weight, socioeconomic factors, access to healthcare, genetic risk, and other factors. Patients with these disorders are at an increased risk of cardiovascular disease, cancer, and other diseases associated with significant morbidity and mortality. In the U.S., there are ~13.8 million type 2 diabetics, 5 million undiagnosed diabetics, and 41 million persons with prediabetes/metabolic syndrome [11–13]. Obesity is a frequent comorbidity, often preceding diabetes by years or decades.

Energy-sensing systems are integral to maintaining homeostasis in normal and transformed cells. Energy deprivation is frequent in cancer cells due to an inadequate vascular supply to meet the needs of increased cell replication. In energy-stressed cells, AMPK is allosterically modified by binding to AMP and ADP, rendering them targetable by AMPK kinases. AMPK activation induces signaling, upregulates energy production, and inhibits energy programming for cell growth and motility. In cancerous cells, this shift often fails to occur even with stress. As a result, cancer cells typically prioritize replication and motility to favor cancer growth and metastasis. Drugs that activate AMPK, most notably metformin, reengage the AMPK failsafe to inhibit proliferation and motility. Thus, metformin provides a unique and generally less-toxic approach to combat the emergence or growth of cancers through inhibition of cell replication. This is particularly important for patients with obesity and type 2 diabetes, who lack homeostasis and experience wide swings in systemic glucose, insulin, and other energy precursors and growth factors that contribute to systemic energy stress.

2. Metabolic dysregulation, breast cancer, and metformin

Abundant epidemiologic and clinical data have shown that obesity and type 2 diabetes increase the risk and severity of cardiovascular disease and human cancer. Each of these chronic metabolic disorders as a single variable significantly increases the risk of breast cancer (BC) [10, 14]. In combination, the risk is increased by 20–50%, depending on the severity of disease and other variables. It is highest in women with abdominal (central) obesity in the postmenopausal setting, in women of all ethnic backgrounds [15–17]. Obesity also promotes BC in premenopausal women of color, especially African Americans and Latinos [18–23]. In patients with obesity and diabetes, BC also presents at a higher disease stage and is more resistant to treatment, resulting in a shorter disease-free interval and a significantly higher mortality rate [24, 25].

Steroid receptor-positive BC (luminal A) and basal (triple negative) BC cells are the most responsive to extracellular glucose at or above 7 mM of glucose to promote cell replication, tumor growth, and motility. In contrast, steroid receptor-positive BC cells that also express high HER2 (luminal B) and steroid receptor-negative, HER2 positive (the HER2 subtype) are less responsive to hyperglycemia, even at levels associated with untreated type 2 diabetes (10 mM glucose or higher) [26].

Glucose directly promotes signaling in epithelial cells or can act indirectly by interacting with molecular signaling proteins, such as the insulin-like growth factor (IGF-1), sex hormones, and adipokines [3, 27, 28]. Insulin and insulin-like growth factors are frequently increased in newly diagnosed BC patients [28–31]. These potent growth factors promote BC growth and are associated with a worse prognosis, both in overweight and ‘normal’ weight women [24, 29, 32–34]. Epidemiological and clinical data show that obesity and type 2 diabetes are particularly associated with luminal A (estrogen and progesterone responsive) as well as triple negative BCs [19, 21, 27, 35, 36].

Metformin (N', N'-dimethylbiguanide) is the most frequently used drug to treat patients with metabolic syndrome (prediabetes) and type 2 diabetes worldwide. It has been used successfully for over six decades and has a very favorable benefit-risk profile [37]. Metformin is stable at room temperature with a long shelf life, is inexpensive and orally administered, and has low rates of significant toxicity or drug-drug interaction. Metformin is best known for its effects on liver and skeletal muscle cells, where it downregulates insulin resistance, lowers serum insulin, stimulates insulin receptor tyrosine kinase activity, inhibits hepatic glucose output (thus lowering A1C), increases glucose uptake by skeletal muscle cells, and can alter fatty acid metabolism.

Epidemiologic data show a significant lowering of cancer risk in patients with metabolic dysregulation (obesity, diabetes, or metabolic syndrome) who take metformin [29, 34, 38, 39]. Metformin use by BC patients has also been associated with improved treatment response and survival. In one meta-analytic study of BC patients with diabetes, metformin use was associated with a 65% improvement in BC-specific survival as compared to nonusers [40]. The anticancer properties of metformin are in contrast to other antidiabetic agents, including sulfonylureas and insulin, which promote cancer growth [9].

It is also taken for its ‘antiaging’ properties in individuals without obesity or metabolic dysregulation, particularly outside of the US [10, 41, 42].

Numerous clinical trials are currently underway in BC patients to evaluate the benefit of metformin combined with or following the administration of other therapeutic agents [29, 32, 33, 43–46]. Studies designed to test the benefit of metformin in patients in only specific molecular subtypes of BC have not been performed, although some have looked at molecular cohort interactions as a secondary goal [30, 47–51]. There are limited data on the use of metformin in metabolically ‘normal’ BC patients. However, our preclinical data suggest that metformin is most active in all molecular subtypes with physiological levels of extracellular glucose [26]. This evidence provides a rationale for testing metformin in otherwise healthy BC patients.

3. AMPK-dependent mechanisms of metformin action in BC

Cellular uptake of metformin requires expression and functionality of the organic cation transporter 1 (OCT1) protein, which in some individuals or BCs may be altered (more or less effective in transporting metformin into the cell) by polymorphism or genetic error [52]. Polymorphisms have also been associated with a decrease in metformin efficacy in diabetic patients [53–55]. In BC cells, we have demonstrated that OCT1 expression is associated with the anticancer activity *in vivo* [44]. Once inside the cell, metformin may directly interact with the metabolic sensor AMPK to induce activation, restoring homeostasis and blocking cellular replication and motility under low energy (stress) conditions. The AMPK ‘switch’ is also influenced by the intracellular AMP:ATP ratio, which in turn is influenced by fatty acid oxidation and

glucose metabolism. Thus, metformin can indirectly affect AMPK, through reduction of gluconeogenesis and thus changing of the AMP:ATP ratio. These mechanisms are represented in **Figure 1**. These processes are modulated by P53 status. It is mutated in many BCs, particularly tumors that are high grade, late-stage or nonluminal in subtype. In BCs that are P53 competent, AMPK activation (from metformin or other triggers) upregulates P53 tumor suppressor activity as a downstream target. This induces activation of cell cycle checkpoint proteins, to inhibit cell proliferation [56]. In P53 incompetent cells, AMPK activation from metformin may be less effective through P53 mechanisms. Given the numerous other actions of metformin, as well as the molecular subtype specificity of the drug, we postulated that P53 status alone would not have a major impact on anticancer effects of metformin. We have demonstrated that this is the case in preclinical studies of numerous BC cell lines [57]. In other cells, metformin may induce cell cycle arrest and death through activation of apoptotic pathways and downregulation of p53 [58, 59] or PARP cleavage, especially in triple negative BC [60, 61].

Activation of mTOR-dependent protein synthesis and cell growth (downstream of the PI3K/Akt signaling axis), along with AMPK, provides a robust signaling platform for BC cell growth, proliferation, and chemotherapy resistance. In addition to activating AMPK, metformin inhibits mTOR and downstream signaling components of this critical pathway. Mutation of the PI3K catalytic subunit (PIK3CA) occurs in 20-35% of BCs [62, 63]. Mutation or loss of the tumor suppressor gene PTEN has also been demonstrated in 40% of BC [64, 65]. Metformin can also inhibit gluconeogenesis and mTOR signaling independent of AMPK and the tuberous sclerosis 2 (TSC2) gene in some experimental systems (in hepatic cells that lack AMPK or its kinase, LKB1). In this model system, metformin induces

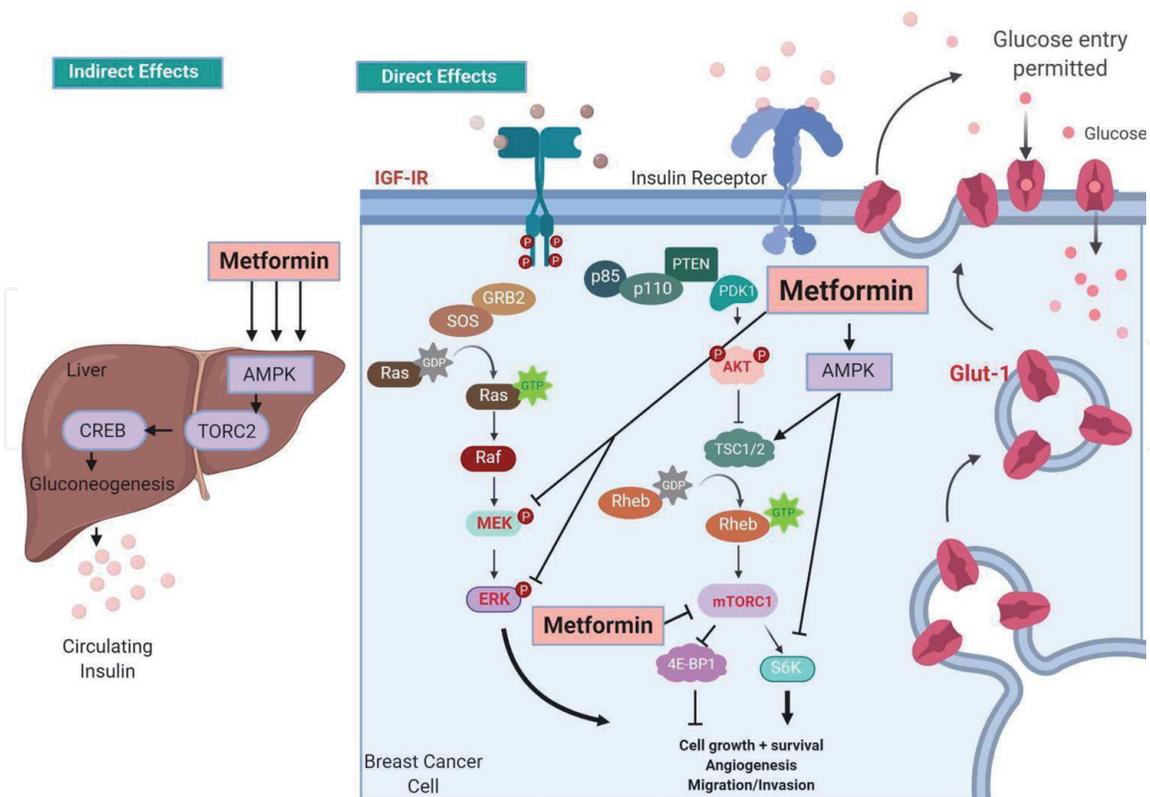


Figure 1.

Metformin AMPK-dependent mechanism of action on breast cancer. Metformin activates AMPK directly through insulin-like growth factor (IGF-I) or insulin receptor, which in turn can activate PI3K/Akt/mTOR or RAS/Raf/MEK/ERK to increase cell growth, survival, angiogenesis, migration, and invasion. Metformin indirectly activates AMPK, which activates mTORC2, CREB, and gluconeogenesis. Lastly, glucose can enter BC cell through GLUT-1, and metformin can directly downregulate GLUT-1 receptor.

downregulation of hepatic gluconeogenesis through non-AMPK-associated mechanisms [66, 67].

Signaling systems and thus metformin sensitivity by dose or mechanism vary by the molecular subtype of BC as well as unique genomic changes in each patient's BC. For example, we have shown that metformin-induced partial S phase arrest increased P-AMPK and reduced P-EGFR, P-MAPK, P-Src, cyclin D1, and cyclin E, with the induction of PARP cleavage and apoptosis only in triple negative BCs [44]. In this tumor subtype, metformin specifically targets Stat3 and is not dependent on mTOR signaling [44]. In non-triple negative BCs (luminal and HER2), metformin induces partial cell cycle arrest at the G1 checkpoint, reduces cyclin D1 and E2F1 expression, and inhibits AMPK, MAPK, Akt, and mTOR activity [44, 57, 68]. Metformin-associated AMPK activation may also inactivate the insulin receptor substrate 1 (IRS1), which in turn regulates IGF-IR and PI3K/Akt signaling pathways to block the pro-growth effects of hyperinsulinemia and insulin-like growth factors typically associated with type 2 diabetes [66, 67, 69].

Metformin is unique in the breadth and complexity of AMPK-dependent direct and indirect targets that inhibit cancer. Several new mechanisms fall into the rapidly expanding field of immuno-oncology. Metformin-induced activation of AMPK activates the programmed death ligand-1 (PDL-1) at S195, reducing stability and membrane localization and thus increasing PDL-1 degradation [70]. Metformin also promotes cytotoxic T cell lymphocyte activity in tumor tissue and enhances tumor-associated immune surveillance [6, 70, 71]. Additionally, metformin upregulates pro-inflammatory cytokines (tumor necrosis factor alpha (TNF α), interleukin-6 (IL-6), IL-1 β , the nuclear factor kappa-light-chain-enhancer of activated B-cells (NF- κ B), the hypoxia-inducible factor 1-alpha (HIF-1 α), and the vascular endothelial growth factor (VEGF), reviewed in [72, 73]).

AMPK-dependent mechanisms of action have been validated using clinical trial-derived BC samples as well as preclinical model systems, reviewed in detail elsewhere [43]. Some of these were especially important to spur the expansion of metformin use in BC patients. The timing, dose, and duration of metformin treatment in BC patients with or without other chemotherapy are actively under investigation. Neoadjuvant metformin, in particular, has shown benefit with a higher rate of complete pathological response, as compared to similar BC patients [74].

3.1 Metformin targets cell cycle proteins in AMPK-dependent manner in breast cancer

AMPK plays an integral role in the regulation of cell cycle and cell division. The ability of metformin to activate AMPK thus has a significant inhibitory effect on cell-cycle associated proteins. This mechanism is represented in **Figure 2**. Expression profiling of BC derived from metformin-treated patients as compared to controls has shown consistent downregulation of many gene encoding proteins involved in mitosis, including kinesins, tubulins, histones, Aurora, as well as Polo-like kinases and ribosomal proteins (critical for protein and macromolecular biosynthesis, respectively) [75]. Given the targeted effects of metformin, it is not surprising that its actions are synergistic with drugs like paclitaxel that induce defects in mitotic spindle assembly, chromosome segregation, and cell division. In combination, metformin and paclitaxel dramatically increase the number of cells arrested in G2-M and apoptosis, as compared to either agent alone [76]. Metformin may also induce GO/G1 arrest due to activation of AMPK, downregulation of cyclin D1, and enhanced binding of CDK2 by p27^{Kip1} and p21^{cip1} [60, 61], especially in non-triple negative cells. Some have shown that metformin sensitivity to GO/G1

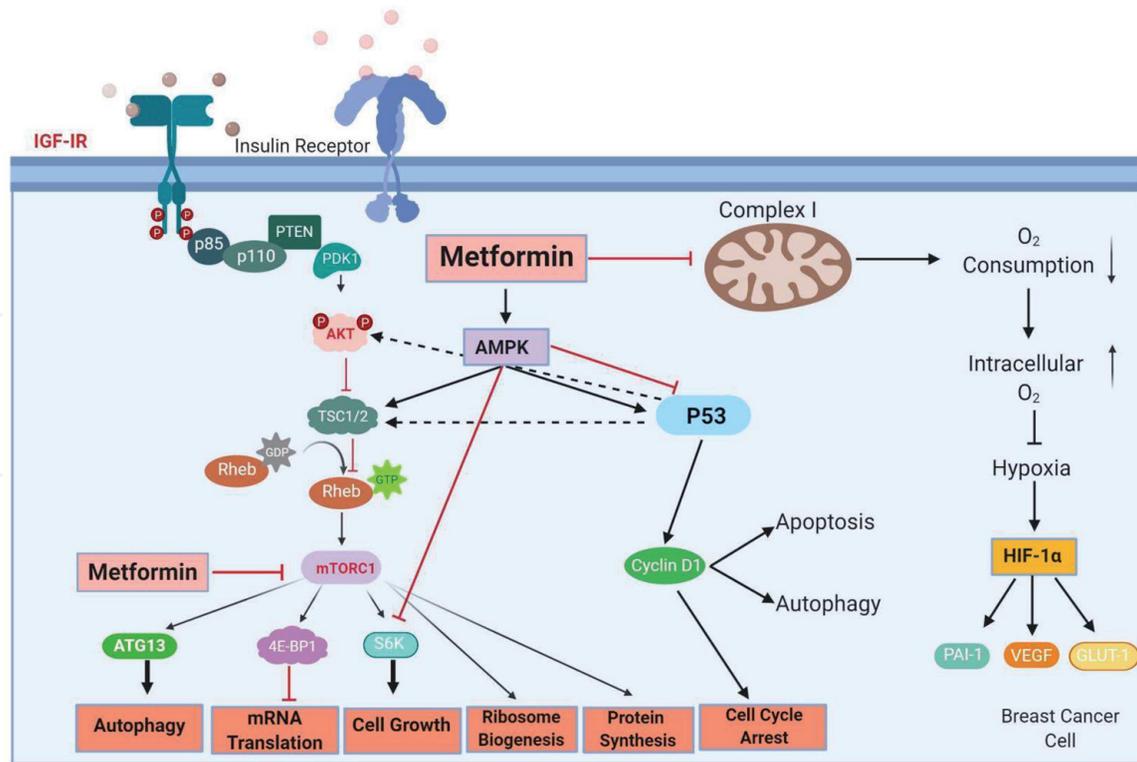


Figure 2.

AMPK-dependent action on cell cycle and alternate mechanisms. Metformin activates AMPK directly through insulin-like growth factor (IGF-I) or insulin receptor, which in turn can activate PI3K/Akt/mTOR or RAS/Raf/MEK/ERK signaling pathway. Metformin can also inhibit downstream signaling intermediates to attenuate autophagy, mRNA translation, cell growth, ribosome biogenesis, protein synthesis, and cell cycle growth. Metformin can also activate AMPK, which blocks P53 and induces cell cycle arrest. Lastly, metformin can block complex I of mitochondrial biogenesis to increase intracellular O₂, which can block HIF-1 and VEGF production.

arrest is linked to overexpression of p27^{Kip1} p21^{cip1} [60, 61]. We have demonstrated that metformin induces cycle arrest at the G1 checkpoint in luminal A, B and HER2 BC [75] associated with a reduction of cyclin D1 and E2F1 expression, with no changes in p27^{Kip1} or p21^{waf1}. While these authors describe how metformin can increase CDK chemical inhibitors to control BC growth [57, 61], others have utilized cell cycle-dependent kinases (CDK) inhibitors with metformin and report that this combination should be used with caution [77].

In addition to downregulating cell replication under stress, metformin upregulates the cellular DNA-damage response, resulting in a decline in the mutational burden for those cancer cells that survive. Mechanisms underlying this effect include selective activation of the ataxia telangiectasia mutated (ATM) gene as well as ATM targets, such as protein kinase CHK2 gene and attenuation of reactive oxygen species ROS that result in DNA damage [78]. Algire et al. have postulated that downregulation of ROS production and thus somatic mutation are likely contributing mechanisms for the reduction in cancer risk associated with metformin use [8].

In summary, AMPK plays a central regulatory role in human cells, including BC where it regulates energy metabolism, cell growth and motility, response to insulin and growth factors, and estrogen production. Metformin induces AMPK activation in a robust manner, to affect numerous target pathways and intermediate molecules. The activity of AMPK and thus metformin can be modified by interacting factors including hormones, growth factors, and energy sensors. Selective targeting of AMPK-dependent pathways has shown less efficacy than metformin alone against BC [79], consistent with the findings that not all mechanisms of metformin action are AMPK dependent.

4. AMP-independent mechanisms of action on metformin

4.1 Metformin action on glucose and metabolism

Upregulation of bioavailable glucose, insulin, and other growth factors increase the risk and promote BC aggression [16, 23, 27, 80, 81]. In addition to shifts in host metabolism, glycolytic reprogramming occurs in breast epithelial cells during malignant transformation. This process is accentuated by systemic dysregulation of carbohydrate and lipid metabolism, as bioavailable sugars and fat typically increase in these patients. Glycolytic reprogramming includes dependence on aerobic respiration, providing less-efficient energy (ATP) production per molecule of glucose from and incomplete oxidative phosphorylation. Cancer cell reprogramming includes activation of numerous signaling intermediaries, including phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), mammalian target of rapamycin (mTOR), phosphatase and tensin homolog (PTEN), and AMPK [82–84]. Changes in other factors including c-MYC, hypoxia-inducible factor 1-alpha (HIF1 α), epidermal growth factor receptor (EGFR), tumor protein 53 (P53), and the Met receptor may also facilitate cancer cell dependence on aerobic glycolysis [16, 85–87].

We have focused on the effects of extracellular glucose and other carbohydrates, combined with or without metformin using BC cell lines and animal models of obesity, metabolic syndrome, and mammary tumorigenesis, summarized in **Figure 3** and detailed elsewhere [26, 47, 49, 52, 57, 68, 88–93]. Importantly, most *in vitro* studies of metformin use commercially purchased media containing ~17 mM glucose (incompatible with human life, above concentrations achieved in diabetes). This is significantly higher than serum derived from normal persons (~5 mM), metabolic syndrome patients (~7 mM), or uncontrolled diabetes (~10 mM) [26]. We have shown that all molecular subtypes of BC cells grown with high glucose media require significantly more metformin to achieve the same anticancer efficacy (i.e., much higher EC₅₀ of metformin) [26]. Normalization of glucose concentration in the culture media significantly reduced the EC₅₀ of metformin for all BC cell types to induce BC growth inhibition or death. This hyperglycemic override of metformin action by dose makes biologic sense, given the ability of glucose to enter cells and promote many of the same pathways we have shown that are critical to metformin action. Similar issues may arise in animal models, particularly if the animals are overfed or obese. In both mouse and rat model systems, we have achieved plasma metformin concentrations equivalent to the normal range in humans, by providing it in the drinking water. We have also shown that metformin accumulates in the cytoplasm, markedly higher than serum levels in mammary tumor cells with functional and sufficient OCT1 protein [26].

Luminal A and some subsets of triple negative BC cell lines show the greatest increase in proliferation when cultured in media with supraphysiologic glucose or insulin. In contrast, luminal B and HER2 BC cells were significantly less responsive to glucose or insulin, even at the highest concentrations examined. This responsiveness pattern was similar to the cellular response to metformin by molecular BC subtype, with triple negative being the most responsive. From a molecular standpoint, triple negative BC cell responsiveness to high glucose and metformin by dose was unique (efficacy at lower EC₅₀s). Triple negative BC cells are especially dependent on glucose/glucosamine (metabolized through glycolysis) and lipids for energy and building block production, cell division, phenotypic aggression, and motility [94]. When grown with media containing supraphysiologic glucose, they upregulate specific genes, including EGFR, P-EGFR, IGF1R, P-IGF1R, IRS2, cyclin D1, and cyclin E expression, and inhibit AMPK/P-AMPK and p38 in a dose-dependent manner [26]. With the addition of metformin, there is a downregulation of these

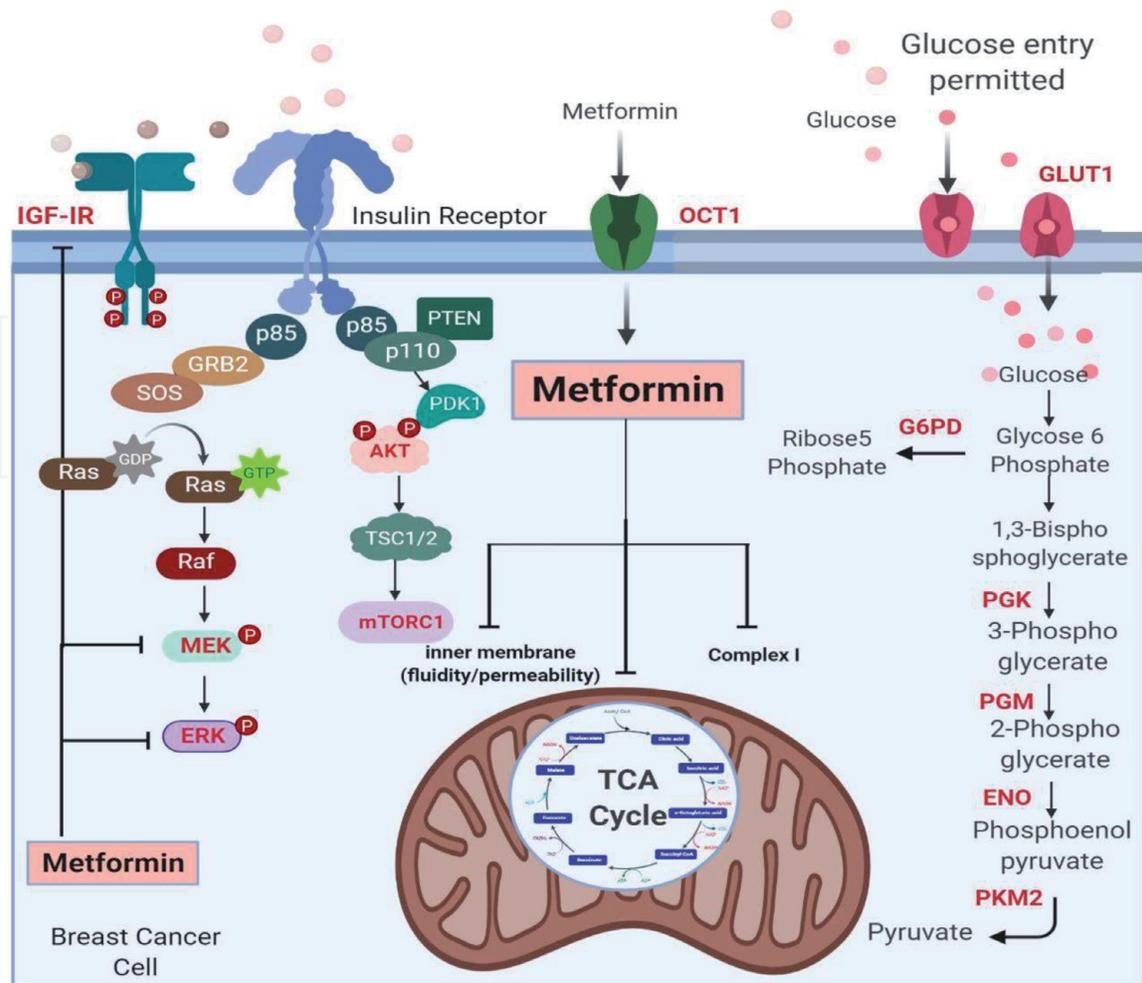


Figure 3.

Metformin action on glucose and metabolism breast cancer. Metformin enters the BC cell through OCT₁ transporter to attenuate inner membrane fluidity/permeability, the Krebs cycle (TCA), and complex I of the mitochondria. Metformin can also block downstream signaling intermediates involved in the PI3K/Akt/mTOR or RAS/Raf/MEK/ERK signaling pathways, which can control BC cell growth. Lastly, metformin blocks GLUT₁ transporter and key enzymes that are involved in carbohydrate synthesis.

genes and the upregulation of genes associated with cell killing and growth control [49, 94]. Our report showed that glucose promotes phenotypic aggression and reduces metformin efficacy by targeting key enzymes that are required for glucose metabolism in TNBC. Such enzymes include G6PD, Fructose-2-6-BP, PGK, PGM, ENO, PKM2, and LDH-A (shown in **Figure 3** and reviewed in [49]). Further, we reported that metformin attenuated the expression of over 20 critical genes involved in glucose metabolism, glucose transporters, gluconeogenesis, and tricarboxylic acid cycle [49]. Metformin-associated gene expression changes also reduced phenotypic aggressiveness and stem-like progenitor cell pool [26, 49, 90, 92]. Metformin treatment also restricted cell proliferation with S phase arrest, motility (through downregulation of intermediate filament proteins), and increased apoptosis (through activation of both the intrinsic and extrinsic pathways) [26, 47, 57, 88, 89, 92]. Metformin significantly inhibits carbohydrate induced pro-oncogenic metabolic and biologic characteristics of triple negative BC cells [26]. Altogether, metformin's ability to target key glucose transporters, such as GLUT₁, along with key genes involved in glucose and carbohydrate metabolism, highlights the role that this agent may play to control highly aggressive malignant BC cells via downregulation of the cellular metabolic machinery.

We have also shown that inhibition of lipid biosynthesis was requisite to the anticancer effects of metformin in triple negative BC cells. It downregulates both

fatty acid synthase (FASN) and the cholesterol biosynthesis pathway, as detailed below. Other studies have focused on interactions between obesity, weight gain, hormonal status, and BC, and more specifically if metformin could be used to disrupt this process. Using a rat model of mammary tumor development after exposure to a carcinogen, animals were overfed and then segregated into lean and obese. Both subsets were subjected to ovary removal, half were given metformin, and they were followed for the development and progression of mammary tumors [52, 93, 95]. Obese rats experienced marked changes in metabolism, akin to metabolic syndrome. Mammary tumors from these obese rats showed enhanced tumor growth and tumor-associated glucose uptake, 50% higher than nonobese rats in association with upregulation of the progesterone receptor. In contrast, the lean rats preferentially deposited excess nutrients in mammary (nontumor) and peripheral tissues. Metformin abrogated systemic metabolic dysregulation, reduced tumorigenesis, tumor progression, and tumor-associated PR expression in obese rats. Similar changes in body weight and obesity are frequent after female menopause has been observed in BC of postmenopausal females with obesity, providing additional clues for the use and timing of metformin associated with BC risk and treatment for future study.

4.2 Metformin action on cholesterol, EGFR signaling, and lipid rafts

The mevalonate pathway, also known as the β -hydroxy β -methylglutaryl-CoA (HMG-CoA) reductase pathway, is critical for cancer cell survival. Inhibition of the pathway by statins or other agents has been shown to have anticancer effects [96, 97]. In contrast, elevated cholesterol has been strongly associated with BC risk, a worse BC-associated outcome and chemotherapeutic resistance. This reflects the pivotal role of lipids including cholesterol in cancer survival and growth, including upregulation of signaling through membrane-bound receptors, facilitation of intracellular signaling pathways, and serving as an anchor for intracytoplasmic filaments to promote motility and invasion and as a precursor for cellular metabolism to generate energy and facilitate replication [98, 99]. We have shown that triple negative BC cells are especially dependent on the upregulation of lipid and cholesterol biosynthesis [100].

Statins are widely prescribed for patients with high cholesterol or lipid abnormalities, most often to reduce the risk of cardiovascular disease. Statins also benefit women to reduce the risk and disease progression of BC. Two population-based studies from Northern Europe are particularly compelling. A Finnish study involving over 30,000 women showed that statin use, pre- or post-BC diagnosis, reduced BC-specific mortality by about 50% [101]. A large Danish study showed a benefit for BC patients as well, with significantly lower recurrence rates in statin users as compared to nonusers. They also reported that lipophilic statins (rather than hydrophilic statins) had the most anti-BC activity [102]. A recent study from MD Anderson Cancer Center suggests that statin use is particularly beneficial for BC patients with triple negative tumors, especially in patients with higher stage disease [95]. Their data are consistent with our preclinical data, showing significant upregulation of lipid metabolism-associated gene triple negative BC as compared to other molecular subtypes. See for further discussion elsewhere [103]. A major issue with statin use is toxicity, which reportedly occurs in up to half of patients. Some statin drugs are also expensive and thus may be unaffordable by many patients.

Metformin, in contrast, is relatively nontoxic and inexpensive. We have demonstrated that metformin has potent effects in lipid and cholesterol biosynthesis in BC cells. More specifically, it inhibits transcriptional activation of HMGCo-A

(the enzyme targeted by statins), as well as over 20 other genes in the cholesterol biosynthesis pathway. We have also shown that it induces translational activation of downstream signaling, including the genes ACAA2, HMGCS1, HMGCR, MVK, MVD, LSS, and DHCR24 (**Figure 4**). Through broad inhibition of cholesterol biosynthesis in triple negative BC, metformin induces a significant reduction of membrane-associated and intracellular cholesterol and reduces GM1 lipid rafts through decreased synthesis and destabilization (disassociation). GM1 lipid raft stability has a profound effect on some receptors that rely on GM1 lipid rafts (like EGFR) for stability, ligand binding, and thus activation, resulting in downstream signaling. We have shown that metformin inhibits cholesterol biosynthesis and raft production, reducing membranous EGFR and its activation associated with downstream signaling in TNBC [91]. We have also shown that in combination, metformin and the statin-mimetic M β CD were synergistic in attenuating cholesterol biosynthesis and cell proliferation [91]. Others have validated our observation that metformin downregulates genes involved in cholesterol biosynthesis, reporting downregulation of HMGCR, LDLR, and SREBP1 [104]. A particularly exciting corollary of these findings is the potential of metformin to synergize with receptor tyrosine kinase inhibitors (RTKIs) against BC. This is an underexplored area of breast oncology research with tremendous translational potential, given the growing use of RTKIs against BC.

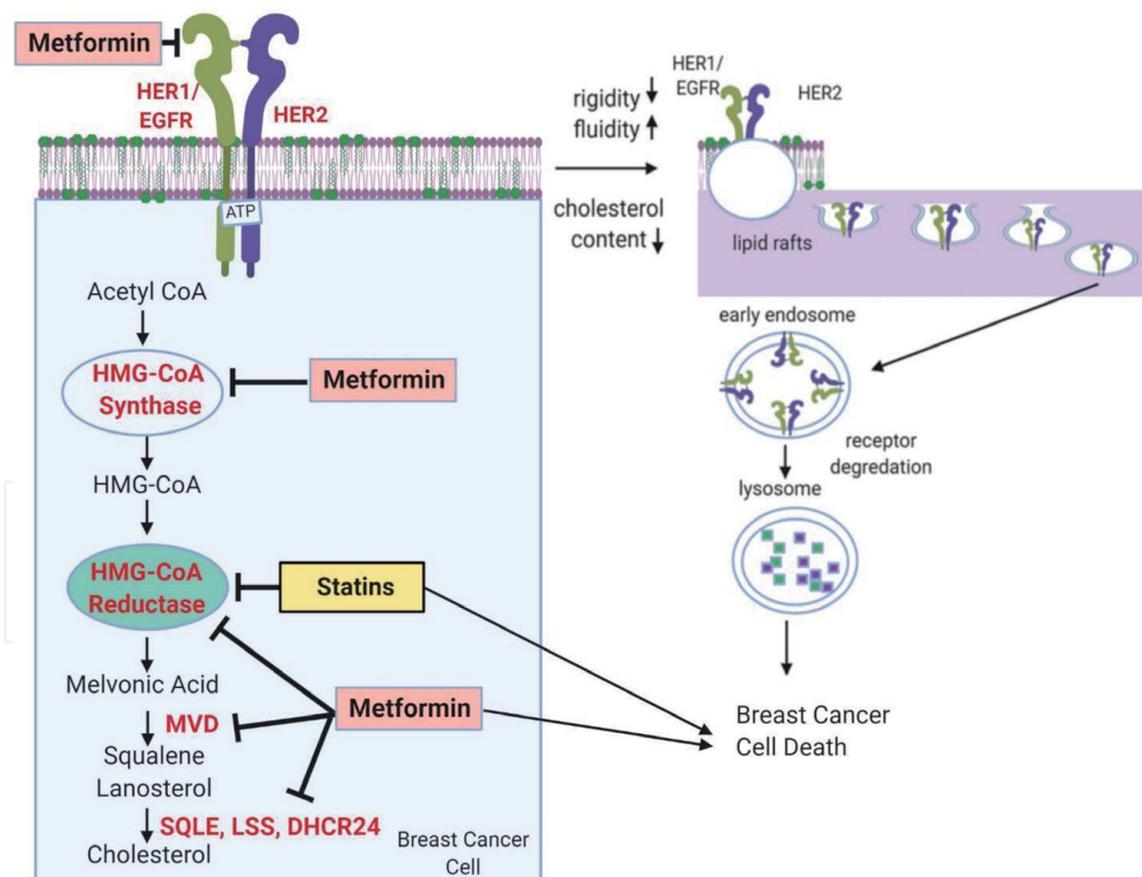


Figure 4.

Metformin action on cholesterol synthesis and lipid rafts. Metformin blocks epidermal growth factor receptor (EGFR), human epidermal growth factor receptors 2/3 (HER2/HER3), which in turn can block key enzymes involved in cholesterol synthesis pathway. Metformin and statins both can inhibit rate limiting step HMG-CoA Reductase, HMGCR. Metformin can also decrease cellular membrane rigidity, increase fluidity, and decrease cholesterol content to allow for the internalization of EGFR, HER2, or HER3 receptors. Internalization of these receptors is through GM1 lipid rafts, which are degraded and allow for BC cell death.

4.3 Metformin action on miRNA and FASN signaling

MiRNAs are endogenous, short (21-25) nucleotide sequences that control gene expression during post-transcriptional translation. It has previously been reported that more than half of human genes are regulated by miRNAs [105]. A growing body of evidence has highlighted the role of miRNAs as master regulators of metabolic processes, such as lipid and cholesterol synthesis [92, 105, 106]. Perturbations of these processes are important for tumor development. Modulation of these regulators using synthetic antagomirs to block the activity of specific miRNAs is an important new area of breast research. Metformin exerts some of its anticancer activity through modulation of miRNAs that target genes in metabolic and other pathways (**Figure 5**) [92, 107, 108]. miRNAs have been reported to be potential biomarkers for BC (i.e., *miR-9*, *miR-10b*, and *miR-17-5p*), whereas others reportedly have prognostic (i.e., *miR-148a* and *miR-335*) or predictive relevance (i.e., *miR-26a*, *miR-30c*, *miR-187*, and *miR-339-5p*) [109].

We have shown that metformin increases several members of the miR-193 family. It upregulates miR-193b, which in turn targets and downregulates the FASN 3'UTR. FASN is an important component of *de novo* fatty acid synthesis. Using an miR-193b mimetic, we induced a drastic reduction in fatty acid synthase (FASN) protein expression as well as increased growth inhibition and apoptosis of TNBC [92]. A separate expression profiling study of metformin-treated TNBC cells has shown similar results [106]. These data show that inhibition of FASN and fatty acid biosynthesis contributes to the potency of metformin against BC cells.

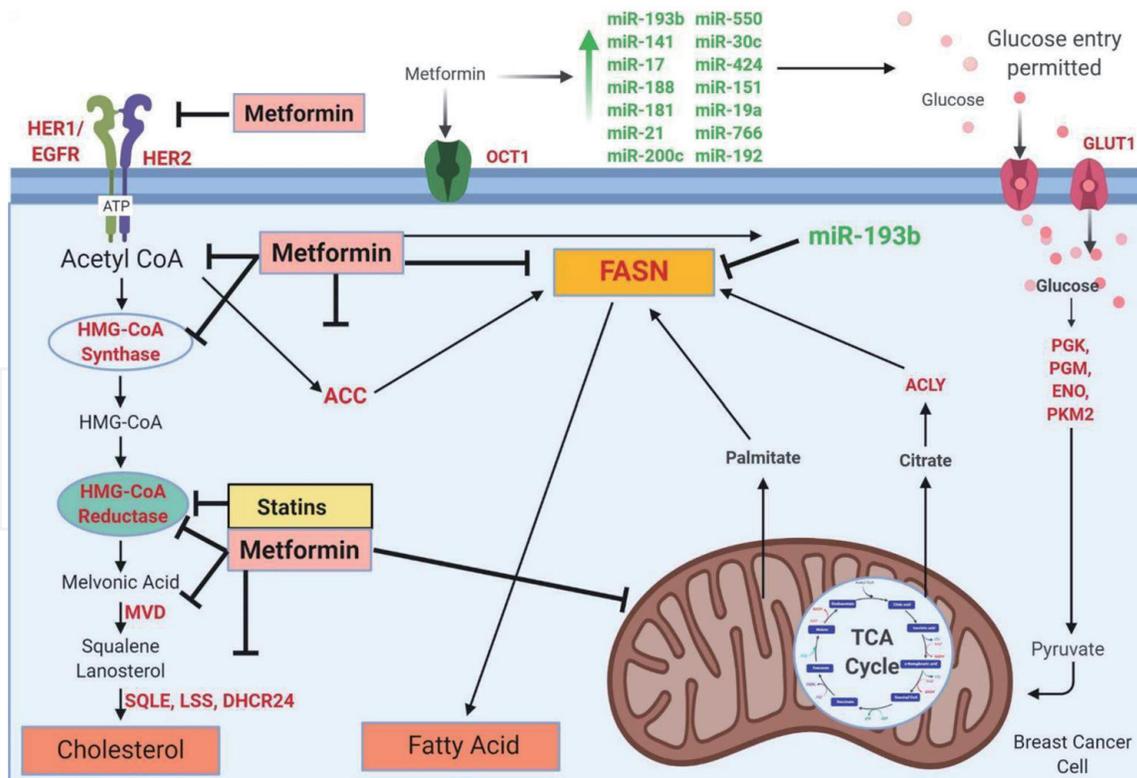


Figure 5. Metformin action on lipid synthesis and miRNAs metformin blocks EGFR, HER2, and HER3, which in turn can block key enzymes involved in cholesterol synthesis pathway as described in **Figure 4**. Metformin can also block acetyl-CoA carboxylase (ACC), which in turn can decrease fatty acid synthase (FASN). Metformin can also increase a myriad of miRNAs (shown in green). One of these miRNAs (*miR-193b*) can target FASN, which can decrease fatty acid synthesis in BC cells. Additionally metformin can block FASN and increase BC cell death.

4.4 Metformin action on PI3K/Akt/mTOR signaling in breast cancer

The PI3K/Akt/mTOR pathway plays a central role in regulating protein synthesis, cell proliferation, tumorigenesis, angiogenesis, tumor growth, and metastasis [63]. While AMPK-dependent phosphorylation is frequently described in metformin-mediated inhibition of the PI3K/Akt/mTOR signaling pathway, AMPK activation is not mandatory for these effects; see schematic in **Figure 6** [57]. We have shown that metformin inhibits Akt and mTOR and inhibits cellular proliferation and colony formation and causes a partial G1 cell cycle arrest in all ER-positive, HER2 normal or abnormal BC cell lines examined [57]. Metformin-mediated inhibition of the PI3K/Akt/mTOR signaling pathway has also been shown to induce inhibition of cell replication, S phase arrest, and apoptosis, with a reduction in E2F1 and cyclin D1 expression in triple negative BC cell lines [57].

4.5 Metformin action in STAT3 signaling

TNBC shows high activation of the signal transducer and activator of transcription 3 (STAT3) signaling pathway, which in turn promotes cell growth, invasion, migration, metastasis, angiogenesis, immune evasion, and drug resistance and inhibits apoptosis [88]. We have shown that metformin specifically targets STAT3 signaling to reduce P-STAT3 at both Ser727 and Tyr705 phosphorylation sites but not STAT3 expression in TNBC, schematically represented in **Figure 6**. In combination with a Stat3 inhibitor, metformin significantly downregulated STAT3

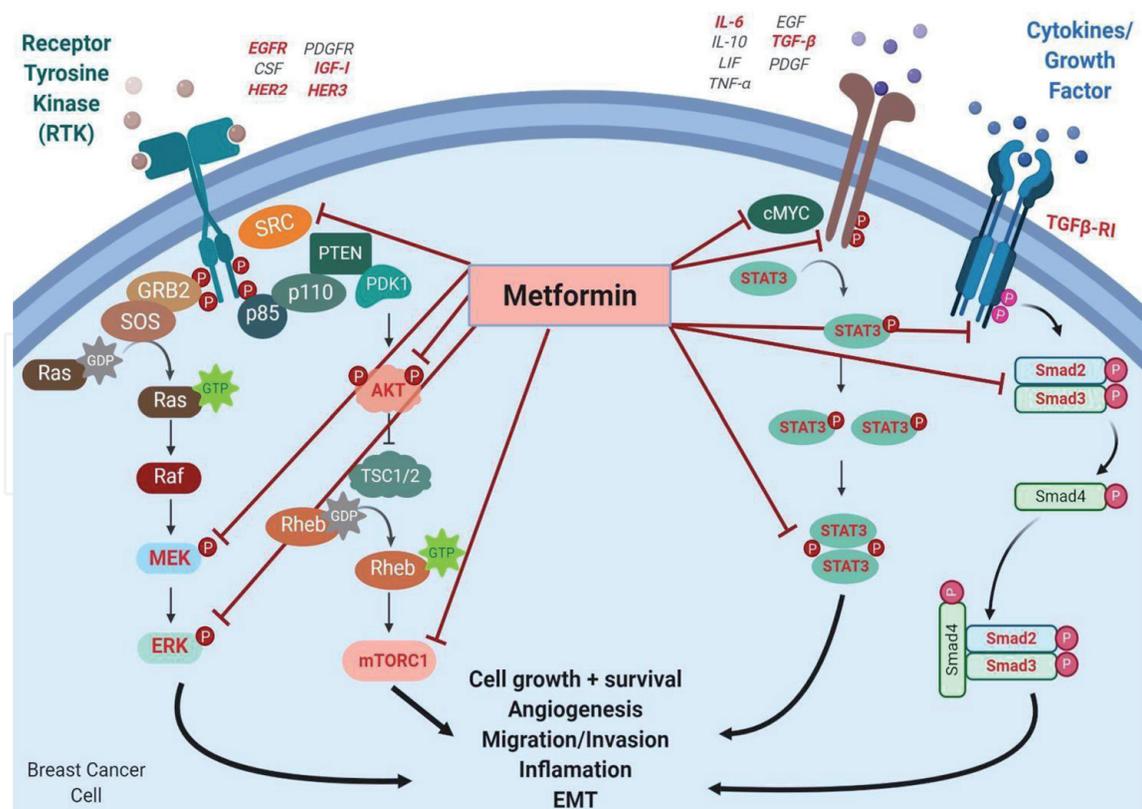


Figure 6.

Metformin action in breast cancer. Metformin can block receptor tyrosine kinase (RTK), such as EGFR, HER2, and HER3. Metformin further blocks downstream signaling intermediates involved in PI3K/Akt/mTOR or RAS/Raf/MEK/ERK signaling pathway, such as AKT, mTOR, MEK, or ERK, which can decrease cell growth, angiogenesis, and migration/invasion. Further, metformin can further block cytokine and growth factor receptors such as the TGF-RII. Metformin can block IL-6/STAT3 pathway and TGF-signaling pathway, which in turn can decrease cell growth, angiogenesis, migration/invasion, inflammation, and EMT.

expression and was synergistic in reducing cell growth and the induction of apoptosis in TNBC [88]. Given that TNBC also shows an upregulation/activation of the PI3K/Akt/mTOR signaling pathways, we then combined metformin with an mTOR inhibitor rapamycin, to determine if it would reduce metformin efficacy. Significant interactions with metformin were not observed; thus, mechanisms underlying its effects are not dependent on mTOR.

The JAK/STAT pathway is upregulated by obesity-associated mechanisms that promote BC growth. Others have demonstrated that metformin attenuates Janus kinase (JAK)/STAT3 signaling at Ser515 and Ser518 within the Src homology 2 domain of JAK1 [110]. Metformin has also been shown to preferentially inhibit nuclear translocation of NK- κ B and phosphorylation of STAT3 in cancer stem cells (CSCs) as compared to non-CSCs [111]. Given the procarcinogenic and prometastatic role that JAK/STAT pathways play in TNBC, the development of therapeutic strategies to attenuate these pathways using metformin may provide benefit with limited toxicity.

4.6 Metformin and TGF- β signaling in TNBC

A subset of TNBC subclassified as mesenchymal-stem like/claudin-low (MSL/CL) characteristically shows high expression and activation of TGF- β signaling, phenotypic aggression, and a worse outcome. In addition to TGF- β receptor 2 expression, BC in this group shows upregulation of Smad2, Smad3, ID1, and ID3 [90]. They are especially responsive to TGF- β ligand 1 (TGF- β 1), resulting in cell proliferation, migration, and invasion. MSL/CL cell lines also demonstrate downregulation of several growth factor receptors in response to metformin, including fibroblast growth factor receptors (FGFR2 and FGFR3), hormone receptors (AR, ESR1, and PGR), and claudin integral membrane proteins of tight junctions (CLDN3, CLDN4, and CLDN7) in the MSL/CL BC subtypes [90]. Metformin directly attenuated TGF- β signaling pathway by downregulating activation of Smad2/Smad3, ID1, and ID3 (**Figure 6**). In combination with TGF- β inhibitors (T β RI-KIs; LY2197s299 or SB431542), metformin synergistically enhanced cell death in MSL/CL BC cells [90]. Overall, these data suggest that targeting TGF- β signaling using metformin with or without a TGF- β inhibitor may provide benefit for patients with MSL/CL BCs.

The process of epithelial-mesenchymal transition (EMT) is also common in TNBC and has been associated with biologic aggression and stem-like properties. Metformin reportedly inhibits EMT in a metastatic canine model of mammary cancer [112]. Others have shown that metformin reduces EMT through blockade of transcription factors like ZEB1, TWIST1, and SNAIL (Slug) [113–115]. Given that TGF- β pathway activation and EMT promote breast cancer stem cells (BCSC), therapeutic resistance, dormancy, and a poor outcome [113], and that metformin has been shown to block these in TNBC, inhibitors against TGF- β -induced EMT combined with metformin may provide benefit in some TNBC patients.

4.7 Metformin action on breast cancer and angiogenesis, and the microenvironment

Clinical studies have demonstrated that diabetic patients treated with metformin are less likely to develop cardiovascular disease, independent of glycemic control. It is unclear whether this outcome reflects downregulation of hyperglycemia and systemic inflammatory triggers or vascular damage, or whether metformin has a direct effect on endothelial cells, vascular resistance, elasticity, and damage

[12, 80, 116]. In the context of breast cancer, it has long been demonstrated that high-stage and grade cancers with a worse prognosis have the capacity to upregulate peri- and intratumoral neo-angiogenesis [117]. The induction of new vessels provides metabolic and oxygen delivery advantages to the cancer cells, facilitating survival and growth. Neo-angiogenesis is also associated with an increased capacity of the BC to metastasize, particularly to distant sites including the visceral organs and brain. We have demonstrated a reduction in vascular density and growth, in association with metformin treatment in preclinical models. Others have shown that metformin is associated with reduced tumor angiogenesis in many different cancer cell types. Metformin and alternate biguanides, such as phenformin, downregulate VEGF-dependent activation of ERK1, inhibiting neo-angiogenesis and reducing microvessel density (MVD) [118]. Wang et al. have shown that metformin also downregulates the expression of two other genes, platelet-derived growth factor B (PDGF-B) and fibroblast growth factor (FGF-2), to reduce angiogenesis [119]. Downregulation of PDGF-B also restricts BC cell proliferation, survival, and migration, [117]. Metformin's effect on the microenvironment and angiogenesis has also been shown to enhance chemo-sensitivity, via a reduction in MVD leakage and cancer cell hypoxia *in vivo* [117]. Thus, metformin's effects go beyond the cancer cell itself and include the peri- and intratumoral microenvironment and neovasculature.

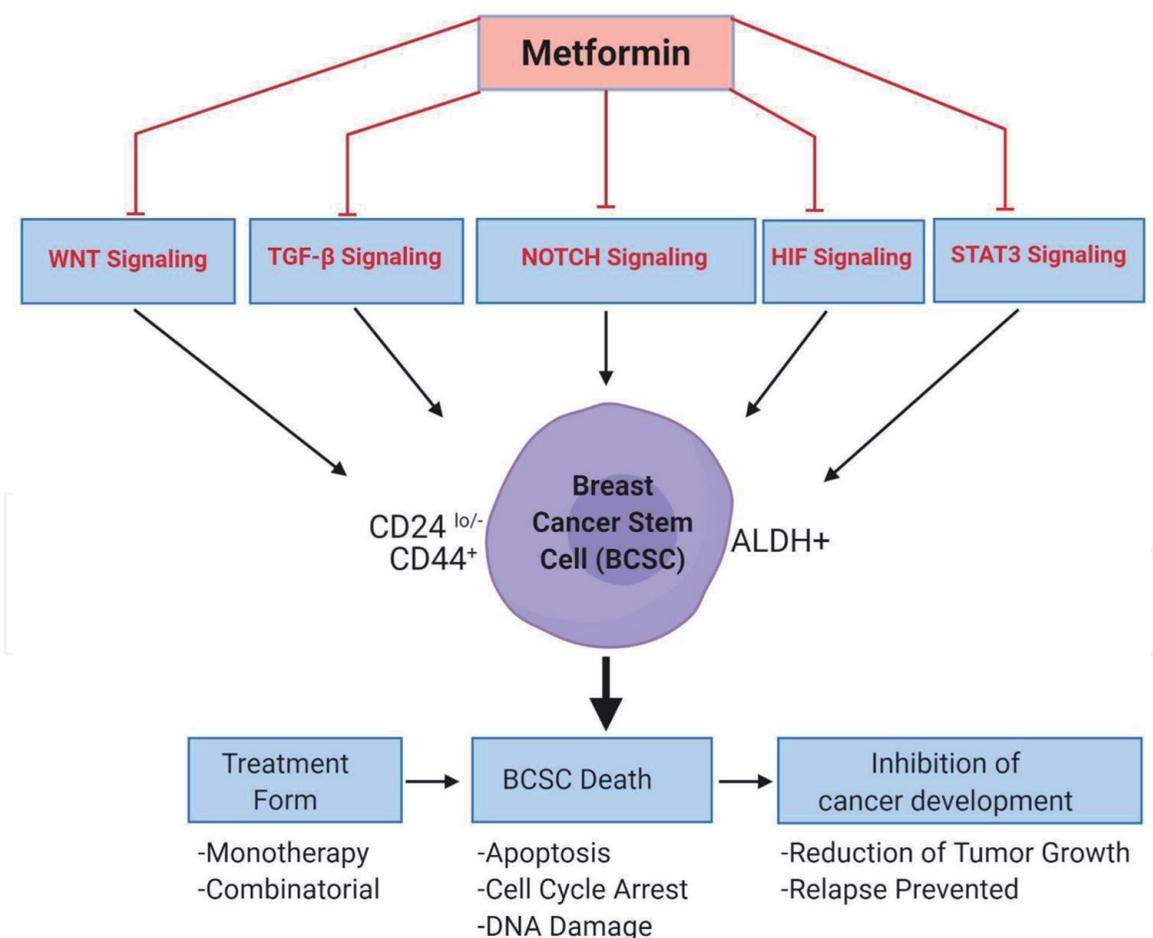


Figure 7.

Metformin action on breast cancer stem cells. Metformin can block a myriad of signaling pathways involved in BCSCs, including WNT, transforming growth factor (TGF), NOTCH, hypoxia inducible factor (HIF), and STAT3 signaling pathways. These pathways are thought to enrich for BCSC through the enrichment of CD44 positive receptor and aldehyde dehydrogenase (ALDH+) and decrease in CD24 expression. Metformin can be given as a monotherapy or combinatorial therapy with alternate chemotherapeutic agents, which in turn can induce BCSC death with an increase in apoptosis, cell cycle arrest, and DNA damage. Overall, reduction in BCSCs can result in reduction of tumor growth and prevention in therapy-mediated relapse.

4.8 Metformin action on breast cancer stem cells

Cancer stem cells (CSCs), also known as tumor-initiating cells (TICs), are the progenitor cells that give rise to BC as well as heterogeneity within transformed populations. CSCs are maintained as a subpopulation within the neoplasm that perpetuates clonal expansion and may facilitate dormancy, metastasis, chemoresistance, and relapse. Among the molecular BC subtypes, TNBC shows the highest enrichment of CSCs, identified by expression patterns with flow cytometry as CD44+, CD24-/low CSC [120]. BC CSCs are particularly sensitive to metformin, which induces rapid cell death facilitated through a number of pathways involved in cell differentiation, renewal, metastasis, and metabolism (**Figure 7**). It directly targets key CSC gene signatures such as Notch 1, NF κ B, Sox2, KLF-4, Oct4, Lin28, MMP-9, and MMP-2 [121]. Metformin attenuates CSCs in resistant BC, through repression of let-7 miRNA [121]. Its ability to attenuate key metabolic genes, such as FASN via upregulation of miR-193b, also contributes to its anti-CSC activity as stem cells are heavily dependent on aerobic glycolysis [92].

The capacity of metformin to induce CSC cell death has significant clinical relevance, given their role in therapeutic resistance, dormancy, and disease progression. Metformin reduces cancer recurrence through the preferential killing of differentiated rather than undifferentiated CSCs [122]. In combination with chemotherapy, metformin is especially active against BC CSCs [111]. In studies of trastuzumab-resistant BC cells as well as xenograft models, the combination of trastuzumab and metformin significantly reduced CD44+, CD24-/low CSC subpopulations and reduced tumor volume [111, 123, 124]. In combination with doxorubicin, paclitaxel, or carboplatin, metformin can also eradicate CSCs and reduce the effective dosage required of the highly toxic chemotherapeutic agents, minimizing patient risk [111, 123].

5. Clinical evidence with metformin in breast cancer prevention and treatment

The pleiotropic oncostatic effects of metformin have been explored as an adjuvant therapeutic option for the management of BC [43, 125, 126]. Epidemiological studies have demonstrated associations between metformin use in patients with type 2 diabetes and decreased cancer incidence and cancer-related mortality [10]. Several observational and randomized trials have evaluated a number of biomarker changes after metformin administration, increasing the footage of metformin as an off-label agent for BC. Over 11 ongoing and 13 completed clinical trials have tested the efficacy of metformin as a monotherapy or in combination with chemotherapy and/or radiotherapy for the management of BC (reviewed in [43, 127]). Goodwin *et al.* have shown that after six months of metformin treatment, a reduction in insulin by 22% had improved metabolic indices, such as insulin sensitivity, body weight, and cholesterol levels in nondiabetic patients with early-stage BC [29]. This information suggests that metformin is effective in the nondiabetic population. These data and other clinical trials further provide support in using metformin as an adjuvant agent as it is the only agent that does not promote BC but actually retards tumor growth. In addition, these clinical trials further support the need to screen for metabolic dysfunction and evaluate whether or not metformin should be integrated into the treatment for BC therapy. Further, BC patients receiving 1500 mg/day of metformin showed a significant reduction in insulin levels and insulin resistance [44, 128]. The effect of metformin in response to neoadjuvant chemotherapy has been examined in diabetic BC patients. This study included 2529

women with BC and confirmed that metformin could achieve higher pathological complete response with neoadjuvant therapy relative to non-metformin users [129]. Dowling et al. have further examined neoadjuvant metformin in a prospective window of opportunity study [32]. Clinical and biological effects of metformin on nondiabetic BC patients were evaluated. These patients were treated with 500 mg of metformin three times daily for 2 weeks. Significant attenuated expression of the insulin receptor was observed in treated breast tumors and had high expression of OCT1 (organic cation transporter 1) [32]. The effect of metformin in nondiabetic BC patients was previously reviewed [43]. Systemic reviews and meta-analyses, highlighting a summary of studies involving metformin therapy in nondiabetic patients and diabetic patients, were reviewed in [43].

5.1 Metformin dose recommended for breast cancer patients

Pharmacokinetic profiling of mouse tumors provided preclinical analysis of appropriate human doses to provide efficient inhibition of tumor growth [130]. Based on this evidence, metformin-mediated activation of AMPK and antitumor function was dependent on cellular uptake of the drug, which is primarily controlled by membrane transporters OCT1, OCT2, and OCT3 [131]. Based on the high expression of OCT transporters, 850 mg/day of metformin is required to inhibit tumor growth efficiently. If a tumor expresses low levels of OCT transporter, then 2250 mg/day is recommended [132]. Additionally, a dose of metformin of 500–850 mg/day is typically recommended with standard chemotherapy (including anthracyclines, platinum, taxanes, and capecitabine) for first- or second-line therapy (please see <https://www.drugbank.ca/drugs/DB00331>). The combination of metformin with a chemotherapeutic agent is recommended for a number of cycles until progression is unacceptable or toxicity develops.

5.2 Indications and contraindications for metformin use for breast cancer

Metformin is not approved for clinical use by the FDA and is still considered investigational for the treatment for BC. While metformin is well established as an inexpensive, well-tolerated, and effective for the treatment of diabetes, adjuvant use of metformin for BC remains to be defined. Current clinical trials have not outlined indications and contraindications for metformin use as adjuvant therapy for BC. Generally, metformin hydrochloride tablets are contraindicated in patients with (1) severe renal impairment (eGFR below 30 mL/min/1.73 m²), (2) hypersensitivity to metformin, and (3) acute or chronic metabolic acidosis including diabetic ketoacidosis. Additionally, current clinical trials with metformin have been listed (<https://clinicaltrials.gov/ct2/show/NCT01310231> and <https://clinicaltrials.gov/ct2/show/NCT01101438>). The NCIC CTG MA.32 Phase III randomized clinical trial has completed enrollment of 3649 nondiabetic women receiving standard surgical, chemotherapeutic, hormonal, biologic, and radiation treatment for T1-3, N0-3, M0 breast cancer. This trial has provided preliminary findings [33] and has not defined clear indications and/or contraindications for metformin use as adjuvant therapy for breast cancer.

6. Conclusions

A preponderance of clinical, epidemiological, and scientific evidence indicates that metabolic dysregulation of carbohydrate and lipid metabolism promote BC pathogenesis and a worse outcome, for women who have the disease [9, 10, 30, 40, 45, 129, 133].

One of the therapeutic agents commonly used in patients with metabolic syndrome or type 2 diabetes, metformin, has demonstrated significant anti-BC activity. Metformin inhibits gluconeogenesis, reduces circulating levels of glucose, increases insulin sensitivity, and reduces hyperinsulinemia associated with insulin [134]. These factors have been associated with BC prognosis. Several mechanisms of metformin action involve AMPK-dependent and AMPK-independent signaling pathways, and these effects are remarkably broad and potent. Its ability to target metabolic dysregulation of carbohydrate and lipid metabolism as well as cancer stem cells appear to be equally important in its anticancer activity against BC [129, 133–137]. Furthermore, the effects of metformin are unique among molecular subsets of BC. A better understanding of these mechanisms will facilitate targeted applications in patients with specific subtypes, fostering the goal of more personalized cancer care.

A number of clinical trials are underway to evaluate metformin in BC patients [30, 44–46, 50, 136]. Most have been designed to evaluate its efficacy, in combination with various chemo- or radiotherapy agents; see (<https://clinicaltrials.gov/ct2/results?term=+cancer+AND+metformin>). Most ongoing or completed clinical trials have evaluated metformin's effect on cellular proliferation or death, pathological response rate, progression-free or overall survival. Some have also sought to compare its efficacy in patients with or without metabolic dysregulation, as a secondary aim. None have specifically been designed to evaluate interactions with CSCs, or in selected molecular subtypes, although correlative studies have provided some data in this regard. The ALTTO trial has shown that metformin improves outcomes for patients with diabetes and either HER2+ or hormone receptor positive BC [30]. The NCIC Clinical Trials Group (NCIC CTG) MA.32 has shown benefit from metformin, as compared to placebo on outcomes in early stage BC [33]. It demonstrated efficacy with improvements in body weight, insulin, glucose, and leptin levels in BC patients examined, regardless of baseline BMI or fasting insulin levels [33].

In conclusion, metformin is a unique drug with a long track record of human use, which has demonstrated robust efficacy against type 2 diabetes and metabolic dysregulation. Epidemiologic data show independent and significant benefit in preventing cardiovascular disease and cancer in these patients. Metformin is an inexpensive oral agent that is currently available worldwide. It is generally well tolerated and has a low risk:benefit ratio. Epidemiological and clinical data have shown that metformin reduces BC incidence and mortality in women with metabolic dysregulation, obesity, and type 2 diabetes. This subpopulation of woman is at significantly higher risk for BC, particularly in the postmenopausal setting. Preclinical and clinical evidence shows that metformin inhibits BC cell replication and tumor growth, decreases tumor aggression, reduces the stem cell pool, and slows motility/metastasis and can promote cell death through apoptosis, autophagy, or upregulation of immunity. Metformin has unique effects on molecular subsets of BC, with the aggressive triple negative BC showing the most sensitivity and lowest EC50 data. TNBC is particularly sensitive to metformin's downregulation of fatty acid and cholesterol biosynthesis, glucose transport, and carbohydrate metabolism. This cancer subtype is typically the most aggressive and is less responsive to traditional chemotherapy; thus, metformin's potency may provide significant benefit especially in these patients.

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Conflict of interest

The authors have declared that no conflict of interest exists.

Abbreviations

ACAA2	Acetyl-coenzyme A acetyltransferase 2
ADP	Adenosine di-phosphate
AKT	Protein kinase B
ALTTO	Adjuvant lapatinib and/or trastuzumab treatment optimization
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
AMPKK	AMP-activated protein kinase kinase
ATP	Adenosine triphosphate
AR	Androgen receptor
BC	Breast cancer
BCSCs	Breast cancer stem cells
CDK	Cyclin-dependent kinase
CL	Claudin-low
CLDN	Claudin integral membrane proteins of tight junctions
CSC	Cancer stem cells
DHCR24	24-dehydrocholesterol reductase
ESR1	Estrogen receptor
HIF-1 α	Hypoxia-inducible factor 1-alpha
EC	Effective concentration/Inhibitory concentration
EGFR	Epidermal growth factor receptor
EMT	Epithelial mesenchymal transition
FASN	Fatty acid synthase
FDA	Food and Drug Administration
FGFR2	Fibroblast growth factor receptor 2
FGFR3	Fibroblast growth factor receptor 3
GLUT1	Glucose transporter 1
GM1	GM1 gangliosidosis marker
HER2	Human epidermal growth factor receptor 2
HER3:	Human epidermal growth factor receptor 3
HMGCo-A	β -Hydroxy β -methylglutaryl-CoA
HMGCS1	Hydroxymethylglutaryl-CoA synthase
HMGCR	3-Hydroxy-3-Methylglutaryl-CoA Reductase
ID1	Inhibitor of differentiation-1
IGFIR	Insulin-like growth factor receptor
IGF1	Insulin-like growth factor-1
IRS1	Insulin receptor substrate 1
IL-1 β	Interleukin 1 beta
IL-6	Interleukin-like 6
JAK	Janus kinase
LDLR	Low-density lipoprotein receptor
LKB1	Liver kinase B1
LSS	Lanosterol synthase
MAPK	Mitogen-activated protein kinases
M β CD	Methyl- β -cyclodextrin
MTOR	Mammalian target of rapamycin
MRCC1	Mitochondrial respiratory-chain complex 1

MSL	Mesenchymal stem-like
MVD	Mevalonate diphosphate decarboxylase
MVK	Mevalonate kinase
NCIC CTG	NCIC Clinical Trials Group
NF- κ B	Nuclear factor kappa-light-chain-enhancer of activated B-cells
OCT1	Organic cation transporter 1
OCT2	Organic cation transporter 2
OCT3	Organic cation transporter 2
P-	Phosphorylated
P53	Tumor protein 53
PARP	Poly (ADP-ribose) polymerase
PGR	Progesterone receptor
PI3K	Phosphatidyl-inositide 3-kinase
PTEN	Phosphatase and tensin homolog
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SRC	Proto-oncogene c-Src
SREBP1	Sterol regulatory element-binding transcription factor 1
STAT3	Signal transducer and activator of transcription 3
TGF- β	Transforming growth factor beta
TNBC	Triple negative breast cancer
TNF- α	Tumor necrosis factor alpha
TSC2	Tuberous sclerosis complex 2
US	United States
VEGF	Vascular endothelial growth factor

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References

- [1] Warburg O. On the origin of cancer cells. *Science*. 1956;**123**(3191):309-314
- [2] Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. *The Journal of General Physiology*. 1927;**8**(6):519-530
- [3] Young CD, Anderson SM. Sugar and fat - that's where it's at: Metabolic changes in tumors. *Breast Cancer Research*. 2008;**10**(1):202
- [4] Warburg O, Geissler AW, Lorenz S. On growth of cancer cells in media in which glucose is replaced by galactose. *Hoppe-Seyler's Zeitschrift für Physiologische Chemie*. 1967;**348**(12):1686-1687
- [5] Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? *Nature Reviews. Cancer*. 2004;**4**(11):891-899
- [6] Violette B et al. Cellular and molecular mechanisms of metformin: An overview. *Clinical Science*. 2012;**122**(6):253-270
- [7] Zhou G et al. Role of AMP-activated protein kinase in mechanism of metformin action. *The Journal of Clinical Investigation*. 2001;**108**(8):1167-1174
- [8] Algire C et al. Metformin reduces endogenous reactive oxygen species and associated DNA damage. *Cancer Prevention Research*. 2012;**5**(4):536-543
- [9] Bowker SL et al. Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin. *Diabetes Care*. 2006;**29**(2):254-258
- [10] Evans JM et al. Metformin and reduced risk of cancer in diabetic patients. *BMJ*. 2005;**330**(7503):1304-1305
- [11] Centers for Disease Control and Prevention. National Diabetes Statistics Report, 2017. Atlanta, GA: Centers for Disease Control and Prevention, U.S. Dept of Health and Human Services; 2017. p. 2017
- [12] Center for Disease Control and Prevention. National Diabetes Fact Sheet: General Information and National Estimates on Diabetes in the United States, 2005. Atlanta: U. S. Department of Health and Human Services, Center for Disease Control and Prevention; 2005
- [13] Bloomgarden ZT. Developments in diabetes and insulin resistance. *Diabetes Care*. 2006;**29**(1):161-167
- [14] Pisanisi P et al. Metabolic syndrome as a prognostic factor for breast cancer recurrences. *International Journal of Cancer*. 2006;**119**(1):236-238
- [15] Vona-Davis L, Howard-McNatt M, Rose DP. Adiposity, type 2 diabetes and the metabolic syndrome in breast cancer. *Obesity Reviews*. 2007;**8**(5):395-408
- [16] Wani B et al. Metabolic syndrome and breast cancer risk. *Indian Journal of Medical and Paediatric Oncology*. 2017;**38**(4):434-439
- [17] Xue F, Michels KB. Diabetes, metabolic syndrome, and breast cancer: A review of the current evidence. *The American Journal of Clinical Nutrition*. 2007;**86**(3):s823-s835
- [18] Schapira DV et al. Abdominal obesity and breast cancer risk. *Annals of Internal Medicine*. 1990;**112**(3):182-186
- [19] Stoll BA. Upper abdominal obesity, insulin resistance and breast cancer risk. *International Journal of Obesity and Related Metabolic Disorders*. 2002;**26**(6):747-753

- [20] Barnett GC et al. Risk factors for the incidence of breast cancer: Do they affect survival from the disease? *Journal of Clinical Oncology*. 2008;**26**(20):3310-3316
- [21] Barnett JB. The relationship between obesity and breast cancer risk and mortality. *Nutrition Reviews*. 2003;**61**(2):73-76
- [22] Fernandez JR, Allison DB. Understanding racial differences in obesity and metabolic syndrome traits. *Nutrition Reviews*. 2003;**61**(9):316-319
- [23] Harvie M, Hooper L, Howell AH. Central obesity and breast cancer risk: A systematic review. *Obesity Reviews*. 2003;**4**(3):157-173
- [24] Lipscombe LL et al. The impact of diabetes on survival following breast cancer. *Breast Cancer Research and Treatment*. 2008;**109**(2):389-395
- [25] Peairs AD, Rankin JW, Lee YW. Effects of acute ingestion of different fats on oxidative stress and inflammation in overweight and obese adults. *Nutrition Journal*. 2011;**10**:122
- [26] Wahdan-Alaswad R et al. Glucose promotes breast cancer aggression and reduces metformin efficacy. *Cell Cycle*. 2013;**12**(24):3759-3769
- [27] Renehan AG, Frystyk J, Flyvbjerg A. Obesity and cancer risk: The role of the insulin-IGF axis. *Trends in Endocrinology and Metabolism*. 2006;**17**(8):328-336
- [28] Pollak MN, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia. *Nature Reviews. Cancer*. 2004;**4**(7):505-518
- [29] Goodwin PJ. Insulin in the adjuvant breast cancer setting: A novel therapeutic target for lifestyle and pharmacologic interventions? *Journal of Clinical Oncology*. 2008;**26**(6):833-834
- [30] Sonnenblick A et al. Impact of diabetes, insulin, and metformin use on the outcome of patients with human epidermal growth factor receptor 2-positive primary breast cancer: Analysis from the ALTTO phase III randomized trial. *Journal of Clinical Oncology*. 2017;**35**(13):1421-1429
- [31] Christopoulos PF, Msaouel P, Koutsilieris M. The role of the insulin-like growth factor-1 system in breast cancer. *Molecular Cancer*. 2015;**14**:43
- [32] Dowling RJ et al. Changes in insulin receptor signaling underlie neoadjuvant metformin administration in breast cancer: A prospective window of opportunity neoadjuvant study. *Breast Cancer Research*. 2015;**17**:32
- [33] Goodwin PJ et al. Effect of metformin vs placebo on and metabolic factors in NCIC CTG MA.32. *Journal of the National Cancer Institute*. 2015;**107**(3):djv006
- [34] Jiralerspong S, Goodwin PJ. Obesity and breast cancer prognosis: Evidence, challenges, and opportunities. *Journal of Clinical Oncology*. 2016;**34**(35):4203-4216
- [35] Alokail MS et al. Combined effects of obesity and type 2 diabetes contribute to increased breast cancer risk in premenopausal women. *Cardiovascular Diabetology*. 2009;**8**:33
- [36] Li YR, Ro V, Tchou JC. Obesity, metabolic syndrome, and breast cancer: From prevention to intervention. *Current Surgery Reports*. 2018;**6**(3):7
- [37] Wang YW et al. Metformin: A review of its potential indications. *Drug Design, Development and Therapy*. 2017;**11**:2421-2429
- [38] Michels KB et al. Type 2 diabetes and subsequent incidence of breast cancer in the Nurses' health study. *Diabetes Care*. 2003;**26**(6):1752-1758

- [39] Chlebowski RT et al. Diabetes, metformin, and breast cancer in postmenopausal women. *Journal of Clinical Oncology*. 2012;**30**(23):2844-2852
- [40] Xu H et al. Metformin use is associated with better survival of breast cancer patients with diabetes: A meta-analysis. *The Oncologist*. 2015;**20**(11):1236-1244
- [41] Hede K. Doctors seek to prevent breast cancer recurrence by lowering insulin levels. *Journal of the National Cancer Institute*. 2008;**100**(8):530-532
- [42] Barzilai N et al. Metformin as a tool to target aging. *Cell Metabolism*. 2016;**23**(6):1060-1065
- [43] Roshan MH, Shing YK, Pace NP. Metformin as an adjuvant in breast cancer treatment. *SAGE Open Medicine*. 2019;**7**:2050312119865114
- [44] Hadad S et al. Evidence for biological effects of metformin in operable breast cancer: A pre-operative, window-of-opportunity, randomized trial. *Breast Cancer Research and Treatment*. 2011;**128**(3):783-794
- [45] Kalinsky K et al. Presurgical trial of metformin in overweight and obese patients with newly diagnosed breast cancer. *Cancer Investigation*. 2014;**32**(4):150-157
- [46] Bonanni B et al. Dual effect of metformin on breast cancer proliferation in a randomized presurgical trial. *Journal of Clinical Oncology*. 2012;**30**(21):2593-2600
- [47] Liu B et al. Metformin induces unique biological and molecular responses in triple negative breast cancer cells. *Cell Cycle*. 2009;**8**(13):2031-2040
- [48] Varghese S et al. High glucose represses the anti-proliferative and pro-apoptotic effect of metformin in triple negative breast cancer cells. *Biomolecules*. 2019;**9**(1):16
- [49] Wahdan-Alaswad RS et al. Metformin targets glucose metabolism in triple negative breast cancer. *Journal of Oncology Translational Research*. 2018;**4**(1):E16
- [50] Martin-Castillo B et al. A phase 2 trial of neoadjuvant metformin in combination with trastuzumab and chemotherapy in women with early HER2-positive breast cancer: The METTEN study. *Oncotarget*. 2018;**9**(86):35687-35704
- [51] Kim J et al. Anticancer effect of metformin on estrogen receptor-positive and tamoxifen-resistant breast cancer cell lines. *Oncology Reports*. 2016;**35**(5):2553-2560
- [52] Checkley LA et al. Metformin accumulation correlates with organic Cation transporter 2 protein expression and predicts mammary tumor regression In vivo. *Cancer Prevention Research*. 2017;**10**(3):198-207
- [53] Jin HE et al. Reduced antidiabetic effect of metformin and down-regulation of hepatic Oct1 in rats with ethynylestradiol-induced cholestasis. *Pharmaceutical Research*. 2009;**26**(3):549-559
- [54] Shu Y et al. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *The Journal of Clinical Investigation*. 2007;**117**(5):1422-1431
- [55] Tzvetkov MV et al. The effects of genetic polymorphisms in the organic cation transporters OCT1, OCT2, and OCT3 on the renal clearance of metformin. *Clinical Pharmacology and Therapeutics*. 2009;**86**(3):299-306
- [56] Jones RG et al. AMP-activated protein kinase induces a p53-dependent

metabolic checkpoint. *Molecular Cell*. 2005;**18**(3):283-293

[57] Alimova IN et al. Metformin inhibits breast cancer cell growth, colony formation and induces cell cycle arrest in vitro. *Cell Cycle*. 2009;**8**(6):909-915

[58] Hsieh Li SM et al. Metformin causes cancer cell death through downregulation of p53-dependent differentiated embryo chondrocyte 1. *Journal of Biomedical Science*. 2018;**25**(1):81

[59] Tanaka R et al. Metformin causes G1-phase arrest via Down-regulation of MiR-221 and enhances TRAIL sensitivity through DR5 up-regulation in pancreatic cancer cells. *PLoS One*. 2015;**10**(5):e0125779

[60] Ben Sahra I et al. The antidiabetic drug metformin exerts an antitumoral effect in vitro and in vivo through a decrease of cyclin D1 level. *Oncogene*. 2008;**27**(25):3576-3586

[61] Zhuang Y, Miskimins WK. Cell cycle arrest in metformin treated breast cancer cells involves activation of AMPK, downregulation of cyclin D1, and requires p27Kip1 or p21Cip1. *Journal of Molecular Signaling*. 2008;**3**:18

[62] Bachman KE et al. The PIK3CA gene is mutated with high frequency in human breast cancers. *Cancer Biology & Therapy*. 2004;**3**(8):772-775

[63] McAuliffe PF et al. Deciphering the role of PI3K/Akt/mTOR pathway in breast cancer biology and pathogenesis. *Clinical Breast Cancer*. 2010;**10**(Suppl 3):S59-S65

[64] Sansal I, Sellers WR. The biology and clinical relevance of the PTEN tumor suppressor pathway. *Journal of Clinical Oncology*. 2004;**22**(14):2954-2963

[65] Kechagioglou P et al. Tumor suppressor PTEN in breast cancer:

Heterozygosity, mutations and protein expression. *Anticancer Research*. 2014;**34**(3):1387-1400

[66] Kalender A et al. Metformin, independent of AMPK, inhibits mTORC1 in a rag GTPase-dependent manner. *Cell Metabolism*. 2010;**11**(5):390-401

[67] Gwinn DM et al. AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Molecular Cell*. 2008;**30**(2):214-226

[68] Zhu P et al. Metformin selectively targets tumor-initiating cells in ErbB2-overexpressing breast cancer models. *Cancer Prevention Research*. 2014;**7**(2):199-210

[69] Ben Sahra I et al. Metformin in cancer therapy: A new perspective for an old antidiabetic drug? *Molecular Cancer Therapeutics*. 2010;**9**(5):1092-1099

[70] Cha JH et al. Metformin promotes antitumor immunity via endoplasmic-reticulum-associated degradation of PD-L1. *Molecular Cell*. 2018;**71**(4):606-620 e7

[71] Eikawa S et al. Immune-mediated antitumor effect by type 2 diabetes drug, metformin. *Proceedings of the National Academy of Sciences of the United States of America*. 2015;**112**(6):1809-1814

[72] Ba W et al. Metformin inhibits pro-inflammatory responses via targeting nuclear factor-kappaB in HaCaT cells. *Cell Biochemistry and Function*. 2019;**37**(1):4-10

[73] Kurelac I et al. The multifaceted effects of metformin on tumor microenvironment. *Seminars in Cell & Developmental Biology*. 2019;**98**:90-97

[74] Jiralerspong S et al. Obesity, diabetes, and survival outcomes in a large cohort of early-stage breast

cancer patients. *Annals of Oncology*. 2013;**24**(10):2506-2514

[75] Oliveras-Ferraros C, Vazquez-Martin A, Menendez JA. Genome-wide inhibitory impact of the AMPK activator metformin on [kinesins, tubulins, histones, auroras and polo-like kinases] M-phase cell cycle genes in human breast cancer cells. *Cell Cycle*. 2009;**8**(10):1633-1636

[76] Rocha GZ et al. Metformin amplifies chemotherapy-induced AMPK activation and antitumoral growth. *Clinical Cancer Research*. 2011;**17**(12):3993-4005

[77] Bellet M et al. Palbociclib and ribociclib in breast cancer: Consensus workshop on the management of concomitant medication. *Therapeutic Advances in Medical Oncology*. 2019;**11**:1758835919833867

[78] Vazquez-Martin A et al. The anti-diabetic drug metformin suppresses self-renewal and proliferation of trastuzumab-resistant tumor-initiating breast cancer stem cells. *Breast Cancer Research and Treatment*. 2011;**126**(2):355-364

[79] Zhao H et al. AMP-activated protein kinase and energy balance in breast cancer. *American Journal of Translational Research*. 2017;**9**(2):197-213

[80] Huang Z et al. Dual effects of weight and weight gain on breast cancer risk. *JAMA*. 1997;**278**(17):1407-1411

[81] Nelson HD et al. Risk factors for breast cancer for women aged 40 to 49 years: A systematic review and meta-analysis. *Annals of Internal Medicine*. 2012;**156**(9):635-648

[82] Marbaniang C, Kma L. Dysregulation of glucose metabolism by oncogenes and tumor suppressors in cancer cells. *Asian Pacific*

Journal of Cancer Prevention. 2018;**19**(9):2377-2390

[83] Lien EC, Lyssiotis CA, Cantley LC. Metabolic reprogramming by the PI3K-Akt-mTOR pathway in cancer. *Recent Results in Cancer Research*. 2016;**207**:39-72

[84] Lin DS et al. Inflexibility of AMPK-mediated metabolic reprogramming in mitochondrial disease. *Oncotarget*. 2017;**8**(43):73627-73639

[85] Vaupel P, Schmidberger H, Mayer A. The Warburg effect: Essential part of metabolic reprogramming and central contributor to cancer progression. *International Journal of Radiation Biology*. 2019;**95**(7):912-919

[86] Chen C et al. Metabolic reprogramming by HIF-1 activation enhances survivability of human adipose-derived stem cells in ischaemic microenvironments. *Cell Proliferation*. 2017;**50**(5):e12363

[87] Shen L et al. Metabolic reprogramming in triple-negative breast cancer through Myc suppression of TXNIP. *Proceedings of the National Academy of Sciences of the United States of America*. 2015;**112**(17):5425-5430

[88] Deng XS et al. Metformin targets Stat3 to inhibit cell growth and induce apoptosis in triple-negative breast cancers. *Cell Cycle*. 2012;**11**(2):367-376

[89] Liu B et al. Potent anti-proliferative effects of metformin on trastuzumab-resistant breast cancer cells via inhibition of erbB2/IGF-1 receptor interactions. *Cell Cycle*. 2011;**10**(17):2959-2966

[90] Wahdan-Alaswad R et al. Metformin attenuates transforming growth factor beta (TGF-beta) mediated oncogenesis in mesenchymal

stem-like/claudin-low triple negative breast cancer. *Cell Cycle*. 2016;**15**(8):1046-1059

[91] Wahdan-Alaswad RS, Edgerton SM, Salem HS, Thor AD. Metformin targets cholesterol biosynthesis pathway, GM1 lipid raft stabilization, EGFR signaling and proliferation in triple negative breast cancers. *Cancer Therapy and Oncology International Journal*. 2018;**9**(3):555765

[92] Wahdan-Alaswad RS et al. Metformin-induced killing of triple-negative breast cancer cells is mediated by reduction in fatty acid synthase via miRNA-193b. *Hormones and Cancer*. 2014;**5**(6):374-389

[93] Giles ED et al. Obesity and overfeeding affecting both tumor and systemic metabolism activates the progesterone receptor to contribute to postmenopausal breast cancer. *Cancer Research*. 2012;**72**(24):6490-6501

[94] Lanning NJ et al. Metabolic profiling of triple-negative breast cancer cells reveals metabolic vulnerabilities. *Cancer & Metabolism*. 2017;**5**:6

[95] Wellberg EA et al. The glucose transporter GLUT1 is required for ErbB2-induced mammary tumorigenesis. *Breast Cancer Research*. 2016;**18**(1):131

[96] Karlic H et al. Inhibition of the mevalonate pathway affects epigenetic regulation in cancer cells. *Cancer Genetics*. 2015;**208**(5):241-252

[97] Nilsson S, Huelsenbeck J, Fritz G. Mevalonate pathway inhibitors affect anticancer drug-induced cell death and DNA damage response of human sarcoma cells. *Cancer Letters*. 2011;**304**(1):60-69

[98] Chimento A et al. Cholesterol and its metabolites in tumor growth: Therapeutic potential of statins

in cancer treatment. *Frontiers in Endocrinology*. 2018;**9**:807

[99] Ding X et al. The role of cholesterol metabolism in cancer. *American Journal of Cancer Research*. 2019;**9**(2):219-227

[100] Ehmsen S et al. Increased cholesterol biosynthesis is a key characteristic of breast cancer stem cells influencing patient outcome. *Cell Reports*. 2019;**27**(13):3927-3938 e6

[101] Murtola TJ et al. Statin use and breast cancer survival: A nationwide cohort study from Finland. *PLoS One*. 2014;**9**(10):e110231

[102] Ahern TP et al. Statin prescriptions and breast cancer recurrence risk: A Danish nationwide prospective cohort study. *Journal of the National Cancer Institute*. 2011;**103**(19):1461-1468

[103] Borgquist S et al. Statins: A role in breast cancer therapy? *Journal of Internal Medicine*. 2018;**284**(4):346-357

[104] Sharma A et al. Metformin exhibited anticancer activity by lowering cellular cholesterol content in breast cancer cells. *PLoS One*. 2019;**14**(1):e0209435

[105] Bajan S, Hutvagner G. Regulation of miRNA processing and miRNA mediated gene repression in cancer. *MicroRNA*. 2014;**3**(1):10-17

[106] Yerukala Sathipati S, Ho SY. Identifying a miRNA signature for predicting the stage of breast cancer. *Scientific Reports*. 2018;**8**(1):16138

[107] Cioce M et al. Metformin-induced metabolic reprogramming of chemoresistant ALDHbright breast cancer cells. *Oncotarget*. 2014;**5**(12):4129-4143

[108] Pulito C et al. microRNAs and cancer metabolism reprogramming:

the paradigm of metformin. *Annals of Translational Medicine*. 2014;**2**(6):58

[109] Bertoli G, Cava C, Castiglioni I. MicroRNAs: New biomarkers for diagnosis, prognosis, therapy prediction and therapeutic tools for breast cancer. *Theranostics*. 2015;**5**(10):1122-1143

[110] Rutherford C et al. Phosphorylation of Janus kinase 1 (JAK1) by AMP-activated protein kinase (AMPK) links energy sensing to anti-inflammatory signaling. *Science Signaling*. 2016;**9**(453):ra109

[111] Hirsch HA, Iliopoulos D, Struhl K. Metformin inhibits the inflammatory response associated with cellular transformation and cancer stem cell growth. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;**110**(3):972-977

[112] Leonel C et al. Inhibition of epithelial-Mesenchymal transition and metastasis by combined TGFbeta knockdown and metformin treatment in a canine mammary cancer Xenograft model. *Journal of Mammary Gland Biology and Neoplasia*. 2017;**22**(1):27-41

[113] Vazquez-Martin A et al. Metformin regulates breast cancer stem cell ontogeny by transcriptional regulation of the epithelial-mesenchymal transition (EMT) status. *Cell Cycle*. 2010;**9**(18):3807-3814

[114] Cufi S et al. Metformin against TGFbeta-induced epithelial-to-mesenchymal transition (EMT): From cancer stem cells to aging-associated fibrosis. *Cell Cycle*. 2010;**9**(22):4461-4468

[115] Oliveras-Ferraro C et al. Micro(mi)RNA expression profile of breast cancer epithelial cells treated with the anti-diabetic drug metformin: Induction of the tumor suppressor miRNA let-7a and suppression of the TGFbeta-induced

oncomiR miRNA-181a. *Cell Cycle*. 2011;**10**(7):1144-1151

[116] Li C, Kong D. Cancer risks from diabetes therapies: Evaluating the evidence. *Pharmacology & Therapeutics*. 2014;**144**(1):71-81

[117] Kannarkatt J et al. Metformin and angiogenesis in cancer - revisited. *Oncology*. 2016;**91**(4):179-184

[118] Orecchioni S et al. The biguanides metformin and phenformin inhibit angiogenesis, local and metastatic growth of breast cancer by targeting both neoplastic and microenvironment cells. *International Journal of Cancer*. 2015;**136**(6):E534-E544

[119] Wang JC et al. Metformin inhibits metastatic breast cancer progression and improves chemosensitivity by inducing vessel normalization via PDGF-B downregulation. *Journal of Experimental & Clinical Cancer Research*. 2019;**38**(1):235

[120] Al-Hajj M et al. Prospective identification of tumorigenic breast cancer cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2003;**100**(7):3983-3988

[121] Bao B et al. Metformin may function as anti-cancer agent via targeting cancer stem cells: The potential biological significance of tumor-associated miRNAs in breast and pancreatic cancers. *Annals of Translational Medicine*. 2014;**2**(6):59

[122] Saini N, Yang X. Metformin as an anti-cancer agent: Actions and mechanisms targeting cancer stem cells. *Acta Biochimica et Biophysica Sinica*. 2018;**50**(2):133-143

[123] Iliopoulos D, Hirsch HA, Struhl K. Metformin decreases the dose of chemotherapy for prolonging tumor remission in mouse xenografts involving

multiple cancer cell types. *Cancer Research*. 2011;**71**(9):3196-3201

[124] Cufi S et al. Metformin-induced preferential killing of breast cancer initiating CD44+CD24-/low cells is sufficient to overcome primary resistance to trastuzumab in HER2+ human breast cancer xenografts. *Oncotarget*. 2012;**3**(4):395-398

[125] Kheirandish M et al. Anti-cancer effects of metformin: Recent evidences for its role in prevention and treatment of cancer. *Current Drug Metabolism*. 2018;**19**(9):793-797

[126] Quinn BJ et al. Repositioning metformin for cancer prevention and treatment. *Trends in Endocrinology and Metabolism*. 2013;**24**(9):469-480

[127] De A, Kuppusamy G. Metformin in breast cancer: Preclinical and clinical evidence. *Current Problems in Cancer*. 2019:100488. DOI: 10.1016/j.currproblcancer.2019.06.003. [Epub ahead of print]

[128] Hadad SM et al. Evidence for biological effects of metformin in operable breast cancer: Biomarker analysis in a pre-operative window of opportunity randomized trial. *Breast Cancer Research and Treatment*. 2015;**150**(1):149-155

[129] Jiralerspong S et al. Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. *Journal of Clinical Oncology*. 2009;**27**(20):3297-3302

[130] Dowling RJ et al. Metformin pharmacokinetics in mouse tumors: Implications for human therapy. *Cell Metabolism*. 2016;**23**(4):567-568

[131] Graham GG et al. Clinical pharmacokinetics of metformin. *Clinical Pharmacokinetics*. 2011;**50**(2):81-98

[132] Cai H, Everett RS, Thakker DR. Efficacious dose of metformin for breast cancer therapy is determined by cation transporter expression in tumours. *British Journal of Pharmacology*. 2019;**176**(15):2724-2735

[133] Tang GH et al. Association of Metformin with breast cancer incidence and mortality in patients with type II diabetes: A GRADE-assessed systematic review and meta-analysis. *Cancer Epidemiology, Biomarkers & Prevention*. 2018;**27**(6):627-635

[134] Cusi K, Consoli A, DeFronzo RA. Metabolic effects of metformin on glucose and lactate metabolism in noninsulin-dependent diabetes mellitus. *The Journal of Clinical Endocrinology and Metabolism*. 1996;**81**(11):4059-4067

[135] Chae YK et al. Repurposing metformin for cancer treatment: Current clinical studies. *Oncotarget*. 2016;**7**(26):40767-40780

[136] Monami M, Dicembrini I, Mannucci E. Thiazolidinediones and cancer: Results of a meta-analysis of randomized clinical trials. *Acta Diabetologica*. 2014;**51**(1):91-101

[137] Qu C et al. Metformin reverses multidrug resistance and epithelial-mesenchymal transition (EMT) via activating AMP-activated protein kinase (AMPK) in human breast cancer cells. *Molecular and Cellular Biochemistry*. 2014;**386**(1-2):63-71