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Metformin and cancer

Doses, mechanisms and the dandelion and hormetic phenomena

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In the early 1970s, Professor Vladimir Dilman originally developed the idea that antidiabetic biguanides may be promising as geroprotectors and anticancer drugs ("metabolic rehabilitation"). In the early 2000s, Anisimov's experiments revealed that chronic treatment of female transgenic HER2-/*neu* mice with metformin significantly reduced the incidence and size of mammary adenocarcinomas and increased the mean latency of the tumors. Epidemiological studies have confirmed that metformin, but not other anti-diabetic drugs, significantly reduces cancer incidence and improves cancer patients' survival in type 2 diabetics. At present, pioneer work by Dilman & Anisimov at the Petrov Institute of Oncology (St. Petersburg, Russia) is rapidly evolving due to ever-growing preclinical studies using human tumor-derived cultured cancer cells and animal models. We herein critically review how the antidiabetic drug metformin is getting reset to metabolically fight cancer. Our current perception is that metformin may constitute a novel "hybrid anti-cancer pill" physically combining both the long-lasting effects of antibodies—by persistently lowering levels of blood insulin and glucose—and the immediate potency of a cancer cell-targeting molecular agent—by suppressing the pivotal AMPK/mTOR/S6K1 axis and several protein kinases at once, including tyrosine kinase receptors such as HER1 and HER2. In this scenario, we discuss the relevance of metformin doses in pre-clinical models regarding metformin's mechanisms of action in clinical settings. We examine recent landmark studies demonstrating metformin's ability to specifically target the cancer-initiating stem cells from which tumor cells develop, thereby preventing cancer relapse when used in combination with cytotoxic chemotherapy (dandelion hypothesis). We present the notion that, by acting as an efficient caloric restriction mimetic, metformin enhanced intrinsic capacity of mitotically competent cells to self-maintenance and repair (hormesis) might trigger counterintuitive detrimental effects. Ongoing chemopreventive, neoadjuvant and adjuvant trials should definitely establish whether metformin's ability to kill the "dandelion root" beneath the "cancer soil" likely exceeds metformin-related dangers of hormesis.

Key words: metformin, cancer, AMPK, stem cells, hormesis

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We all know there are benefits to improving cancer patients' lifestyles through better diet and more exercise. Besides effects on *quality* of life, healthy lifestyle interventions' effects on cancer patients might be also viewed in terms of *quantity* of life. This assumption becomes apparent when considering that essential hallmarks of cancer disease (e.g., uncontrolled proliferation) are intertwined with an altered tumor cell-intrinsic metabolism, either as a consequence or as cause.[1] In this scenario, the implementation of calorie/dietary restriction (i.e., under-nutrition without malnutrition) should be expected to directly regulate several factors intimately implicated in the molecular biology of cancer itself. Yet, we should be conscious that implementation of lifestyle interventions aimed to significantly disrupt signalling pathways and/or energy factories that account for metabolic reprogramming of

tumor cells can be costly and challenging for both patients and practitioners.[2] It is obvious that many will prefer the effortlessness and perceived confidence of being treated with a drug that targets cancer's Achilles metabolic heel.[3] The good news is that an *old* pharmacological approach may notably augment the basic approach of diet and behavioral modifications in helping cancer patients (and, perhaps, on healthy individuals at risk of cancer) to manage their *energy charge* on a daily basis. This drug is metformin, a readily available, inexpensive and generally well tolerated biguanide currently approved for the treatment of non insulin-dependent diabetes mellitus.

Anti-Carcinogenic Metformin: Back to the Old Tricks

50 years after its launch for the treatment of type 2 diabetes, we are now leaving a renaissance of the potential anticancer value of metformin. However, as magnificently recently reviewed by Dr. Berstein,[4] it should be recognized that metformin has long been known to reduce the growth (and perhaps onset) and progression of tumours. In the early 1970s, Professor Vladimir Dilman pioneeringly developed the idea that antidiabetic biguanides may be promising as geroprotectors and anticancer drugs.[5] Using phenethylbiguanide (phenformin), a chemical cousin of metformin, he and co-authors at the N.N. Petrov Research Institute of Oncology (St. Petersburg, Russia) achieved the so-called "metabolic rehabilitation" in breast and colon cancer patients.[6,7] In these patients, phenformin-based clinical management induced retardation of relapses and decrease incidence of primary multiple neoplasias. In animal models, phenformin treatment not only extended lifespan of C3H mice by ~23% but further reduced tumor incidence by 80%.[8-10] In the early 2000s, Anisimov's experiments at the Petrov Institute revealed that chronic metformin treatment of female transgenic HER-2/*neu* mice significantly reduced the incidence and size of mammary adenocarcinomas and increased the mean latency of the tumors.[11,12] Epidemiological studies have confirmed that metformin, but not other anti-diabetic drugs, significantly reduces cancer incidence and improves cancer patients' survival in type 2 diabetics.[13-16]

At present, and due to ever-growing preclinical studies using tumour-derived cultured cancer cells and animal models, the *bench-to-clinic* scenario for metformin and cancer is rapidly evolving. Since insulin and its related growth factors are widely believed to be mitogenic in an important sub-group of cancer patients, and because pre-operational insulinemia associates with breast cancer progression rates, metformin has been proposed as a therapeutic agent for non-diabetic breast cancer patients based largely on its ability to systemically reduce serum insulin and glucose levels.[2,17-19] On the other hand, the ability of metformin to suppress hepatic gluconeogenesis via activation of the fuel gauge adenosine monophosphate-activated protein kinase (AMPK) may explain also its functioning as a general inhibitor of cancer cell growth. Indeed, most researchers in the field have adopted a simplified working model in which metformin exerts anti-tumoral effects by activating AMPK which, in turn, suppresses activity of the mammalian Target Of Rapamycin (mTOR) and lastly decreases activity of the mTOR effector S6K1.[20-24] This dual action of metformin (insulin reduction and mTOR inhibition—a master integrator of cell growth and division in response to cell energy state, nutrient status, and growth factor stimulation)—along with the modulation of several other targets (e.g., p53, p21, Cyclin D1, survivin, Src, etc.,)[25-32] makes it a particularly attractive molecule for evaluation in human malignancies.

Conceptually, metformin could be considered a "hybrid" anticancer compound that physically combines both the long-lasting effects of antibodies—by persistently lowering levels of blood insulin and glucose—and the immediate potency of a cancer cell-targeting molecular agent—by suppressing the pivotal AMPK/mTOR/p70S6K1 axis and several protein kinases at once, including crucial cancer-related tyrosine kinase receptors such as $HER2^-$ (**Fig. 1**). This unexpected "*going from the bedside back to the bench*" of metformin—a readily available, inexpensive and generally well tolerated oral medication—warrants additional studies to definitely evaluate its potential as a new class of antitumor agent. At the time of writing, in response to the inquiry "metformin and cancer", a search in ClinicalTrials.gov—a service of the US NIH that registers federally and privately supported clinical trials conducted in the US and around the world—yields seven open studies evaluating the efficacy and safety of treating cancer patients with metformin (**Table 1**). In addition, at the European Institute of Oncology in Italy, the Division of Cancer Prevention and Genetics is planning a presurgical randomized, double blind, placebo-controlled phase II biomarker trial in which breast cancer patients not suitable for neoadjuvant therapy will be randomly assigned to either metformin (850 mg twice/daily) or placebo tablets (28 ± 7 days) until surgery to evaluate the real activity of metformin on tumour proliferation (as measured by Ki-67).[33] Also in Italy, there are two further on-going randomized controlled clinical trials (RCTs), highly intertwined, on metformin-based primary prevention of breast cancer.[34] First, the Plotina study will evaluate the effect of metformin on breast cancer primary prevention and on primary prevention of cardiovascular diseases, and patients are being randomized to the treatment arm (850 mg twice/daily) or placebo. Second, the Milan Study will follow a similar design (i.e., metformin versus placebo) plus a diet-intervention focus based on the reduction of high caloric/high glycemic index food, an increase in vegetable intake as well as 30 minutes of physical activity per day. With an overall sample size of 16,000 postmenopausal women and a 5-year follow-up period (325 incidents of breast cancer cases have been estimated to occur among the trial participants), the results of these two trials will clarify in a clinical setting the chemopreventive abilities of metformin envisioned in experimental studies.

Metformin and Breast Cancer: A Critical Reappraisal

We enthusiastically agree that planned chemopreventive, neoadjuvant and adjuvant trials with the anti-diabetes

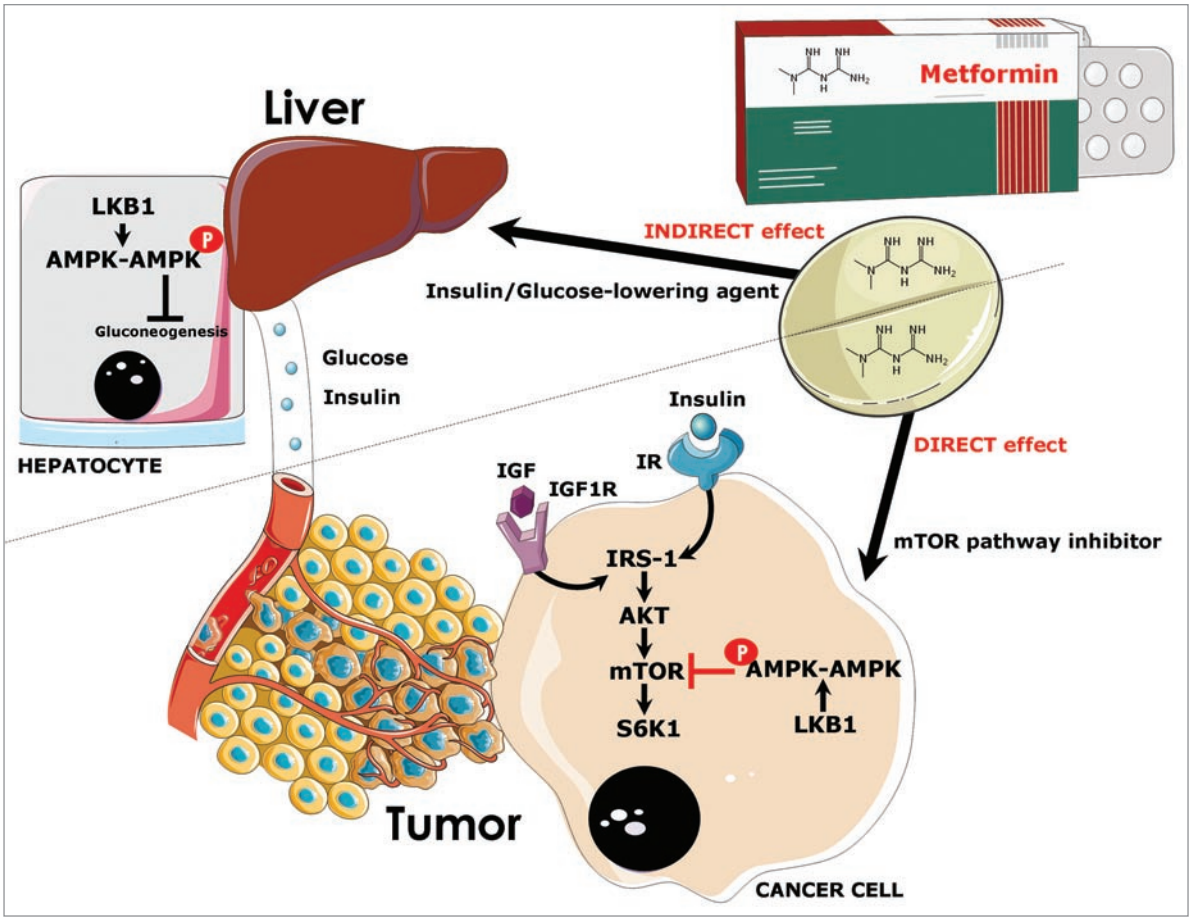

Figure 1. The antidiabetic pill metformin: Getting reset to metabolically fight cancer. The biguanide metformin physically combines the long-lasting effects of antibodies (by persistently lowering levels of blood insulin and glucose) and the immediate potency of a cancer cell-targeting molecular agent (by suppressing the pivotal AMPK/mTOR/S6K1 axis and several protein kinases at once, including HER1, HER2, Src, etc.,). Metformin's unique mechanism of action may provide for a potential double-strike against the anabolism-addicted tumor itself and its crucial insulin/glucose supply.

biguanide metformin in breast cancer should proceed when considering all the clinical and epidemiologic evidence we have accumulated in the last few years. However, we should consider the fact that, as with many other anti-cancer drugs used on a daily chronic basis, exposure to metformin might be a double-edged sword if more aggressive (metformin-refractory) cancer cells emerge.[35] This crucial issue has been previously reached by some authors when revealing that metformin-induced AMPK activation may stimulate neoangiogenesis and tumor growth in xenograft models using the estrogen receptor-negative MDA-MB-435 breast cancer cell line. On the one hand, it was stressed that breast cancer is irrelevant in this case because the MDA-MB-435 is derived from melanoma.[37] On the other hand, some authors have argued that these undesirable effects of metformin solely rely on the supra-clinical doses of metformin employed in pre-clinical studies and, therefore, they are clinically irrelevant.[38] A similar argumentation has been proposed to disregard our recently developed model of acquired auto-resistance to metformin in A431 epidermoid cancer cells.[39] Unlike metformin-naive A431 parental cells, metformin-refractory MetR10 cells distinctively exhibit increased phosphorylation of IGF-1R and Vascular Endothelial Growth Factor Receptor (VEGFR) 3.[40] These findings suggested that disruption of the AMPK/mTOR/S6K1 axis on chronic exposure to metformin efficiently relieves negative feedback suppression on the IGF-1R/IRS-1 axis, leading to elevation of cell survival signals and thus counteracting the antitumor activity of metformin.[40] Because the plasma concentrations of metformin in diabetic patients treated with the drug are estimated to be consistently less than 50 μmol/L, it is easy to calculate that metformin-refractory cancer cells capable to grow in the presence of 10 mmol/L metformin are being exposed to, at least, 200-fold excess over the recommended therapeutic levels. This simple calculation, however, does not entirely weaken the clinico-biological relevance of our and other pre-clinical findings using cultured cancer cells. Rather, it simply imposes one viewpoint, which focuses on the insulin-lowering mode of action as the main mechanism by which metformin may influence the outcome of clinical breast cancer. Jiralerspong and colleagues observed that diabetic breast cancer patients receiving metformin and neoadjuvant chemotherapy had a higher rate of pathological complete responses (pCR) than no diabetics not receiving metformin (i.e., the rate of pCR was 24% in the metformin group, 8.0% in

Table 1. Ongoing metformin-based clinical trials in human solid tumors (*ClinicalTrials.gov*; november 2009)

Condition	Official title	Identifier	Purpose	Primary objective
Breast Cancer (BC)	Clinical and Biologic Effects of Metformin in Early Stage BC	NCT00897884	The study will be testing metformin in patients with BC who are about to undergo surgery. Patients will take metformin 3 times daily for about 2–3 weeks prior to their surgery date. It is hypothesized that metformin will reduce cell proliferation rates in tumor tissue	To determine if taking metformin prior to surgery can reduce cell proliferation rates in tumor tissue. To be determined by tumor specimen analysis using pre- and post-operative biopsy sample
Breast Cancer (BC)	The Impact of Obesity and Obesity Treatments on BC: A Phase I Trial of Exemestane With Metformin and Rosiglitazone for Postmenopausal Obese Women With ER^+ Metastatic BC	NCT00933309	To identify the dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) of the exemestane plus metformin/rosiglitazone combination (phase I).	Dose-limiting toxicity
Breast Cancer Endometrial Cancer Kidney Cancer Lung Cancer Lymphoma Unspecified Adult Solid Tumor	A Phase I Study of Temsirolimus in Combination With Metformin in Advanced Solid Tumors	NCT00659568	This phase I trial is studying the side effects and best dose of metformin when given together with temsirolimus in treating patients with metastatic or unresectable solid tumor or lymphoma	To establish the maximum tolerated dose and recommended phase II dose of metformin hydrochloride when administered with temsirolimus in patients with advanced solid cancers or lymphoma.
Prostate Cancer	A Phase II, Open Label Assessment of Neoadjuvant Intervention With Metformin Against Tumor Expression of Signaling	NCT00881725	This study will investigate the effect of neoadjuvant metformin therapy in the inhibition of growth and proliferation of prostate cancer cells prior to radical prostatectomy	Difference in P-AKT staining
Breast Cancer (BC)	Efficacy and Safety of Adjuvant Metformin for Operable BC Patients	NCT00909506	Adjuvant metformin use in BC patients with overweight or pre-diabetes mellitus (DM) may improve their body condition including weight loss	Weight loss
Breast Cancer (BC)	Pre-Surgical Trial of Metformin in Patients With Operable BC	NCT00984490	This phase I trial is studying how well metformin hydrochloride works in treating women with stage I or stage II BC that can be removed by surgery	To determine the in situ effects of metformin hydrochloride on proliferation (Ki67) and apoptosis (caspase-3) in women with operable stage I or II BC
Breast Cancer (BC)	Pre-Surgical Intervention Study for Evaluating Metformin for BC	NCT00930579	The purpose of this pilot study is to use a pre surgical intervention model to evaluate the biologic effects of metformin in women with newly diagnosed early invasive BC. Metformin is a drug commonly used to treat patients with diabetes. This model will be used to evaluate the effects of metformin	Effects of metformin on AMPK/mTOR signaling pathway.

the nonmetformin group, and 16% in the nondiabetic group).[41] In this regard, it could be argued that the report by Jiralerspong et al. provides evidence that the use of metformin in standard clinical doses may be associated with clinical benefit irrespective of metformin-induced pro-angiogenic effects and/or metformin-triggered mechanisms of acquired autoresistance.[39] However, stated more neutrally, Jiralerspong et al.[41] retrospectively found that the use of metformin in standard clinical doses was associated with clinical benefit in *diabetic* patients (in whom hyperinsulinemia-induced activation of signaling pathways downstream of the insulin receptor in cancer cells could be significant). Therefore, on-going trials of metformin as an adjuvant treatment in breast cancer should elucidate whether the ability of standard clinical metformin doses to efficiently lower circulating levels of insulin can be extrapolated to beneficial effects in non-diabetic breast cancer patients. Moreover, it is just obvious that Jiralerspong et al.[41] did not evaluate the relevance of the AMPK/mTOR → IGF-IR feedback mechanism in the clinical efficacy of metformin (as it was not an end-point of their study).

Metformin and Breast Cancer: Dose versus Mechanism of Action

When reviewing the ever-growing list of manuscripts on the anti-tumor effects of metformin using human tumor-derived neuroblastoma, prostate, breast, ovary, colon, glioma, melanoma and endometrial cancer cell lines cultured in vitro, it becomes obvious that all these studies have been performed by exposing cell cultures to metformin concentrations ranging from 1 to 100 mmol/L. In most of them, insulin-independent, AMPK/mTOR-related statistically significant cytotoxic effects of metformin were observed at concentrations ranging between 5 and 10 mmol/L. Indeed, few of these studies could report any significant activation of AMPK when using metformin concentrations lower than 5 mmol/L. Given that metformin most likely does not directly activate either LKB1 or AMPK as the drug does not influence the phosphorylation status of AMPK by LKB1 in vitro, we might agree that all these pre-clinical evidences from recent biomedical bibliography should be considered experimental artefacts without clinical value. We are then obligated to consider AMPK activation at supra-clinical concentrations of metformin as a metabolic response to "general metformin toxicity". However, it could be difficult to explain why lost or decreased expression of LKB1 as well as specific genetic silencing against the *AMPK* gene can efficiently promote resistance against the anti-proliferative effects of metformin.[20-23] More importantly, there is strong evidence that AMPK activation indeed occurs in response to metformin treatment as a downstream effect on complex I of the mitochondrial electron transport chain.[42-44] In hepatic tissue, as shown by pioneering works of Owen et al.[43] metformin concentrations of 8 mmol/L represent physiologically relevant doses of metformin in hepatic tissue because liver receives the majority of the blood via the portal vein, which may contain concentrations of metformin substantially higher than those present in the general circulation. As suggested by Carvalho et al.[44] the positive charge of metformin could promote its accumulation within the mitochondrial matrix by 1,000-fold (>20 mmol/L). Indeed, it has been reported that metformin accumulates in tissues at concentrations several-fold higher than those in blood,[45] indicating that AMPK-related therapeutically active concentrations of metformin employed in preclinical models (1–10 mmol/L) might be attained also during cancer treatment. Obviously, future studies should elucidate whether retention of metformin by tissues other than liver or small intestine (e.g., breast tissue) may represent deep compartments for the drug. In our laboratory, we carried out step-wise drug selection protocols in which metformin-naive cancer cells were exposed to small, incremental increases of metformin doses that should affect mitochondria functioning to activate AMPK (1.25, 2.5, 5 mmol/L). This stepwise drug selection was continued until the cancer cell population could sustain viability and proliferate when challenged with 10 mmol/L metformin. Similar drug selection schemes have been routinely used to isolate drug-resistant cell lines and identify mechanisms of drug resistance with clinical relevance.

Metformin and Breast Cancer: Dose versus Tumor Cell Compartment (The Dandelion Phenomenon)

When considering a "dose-dependent" scenario, the anti-tumor effects of metformin may unexpectedly depend on the cancer cell compartment of breast carcinomas. A landmark study by Hirsch et al.[46] has recently revealed that tumor-forming, self-renewing cancer stem cells, which are resistant to well-defined chemotherapy, are exquisitely sensitive to metformin. In their hands, low doses of metformin (0.1 or 0.3 mmol/L) likewise failed to significantly affect cell viability in the non-stem population of differentiated cancer cells. Intriguingly, these low concentrations of metformin (still ~10-fold higher than plasma concentrations of metformin in diabetic patients) selectively killed cancer stem cells. Consistently with the "*dandelion hypothesis*", in which both dividing differentiated cancer cells and tumorigenic (stem-like) cancer cells must be targeted to prevent relapse,[47] concurrent treatment with metformin and the cytotoxic doxorubicin was found to reduce tumor mass and prevent relapse much more effectively than either drug alone in a xenografts mouse model.[46] IGF-1 should be considered a potential breast stem cell mitogen and it might be predicted that elevated levels of IGF-1 might trigger expansion of breast stem cell pools. Metformin acting systemically to indirectly lower insulin levels may significantly decrease the number of targets at risk (i.e., breast stem cells) for oncogenic transformation, thereby providing a previously unrecognized molecular explanation to landmark epidemiological studies which demonstrated lower breast cancer mortality in patients treated with metformin and a dose-dependent decrease in breast cancer incidence in metformin-treated diabetics. Although it is unclear what molecular mechanisms control the maintenance and survival of breast cancer stem cells, findings by Hirsch et al.[46] provide a strong rationale for studying yet to be explored roles for insulin, IGF-1/IGF1-R1 and AMPK/mTOR signaling as metformin-targetable pathways beyond Notch, Wnt and Hedgehog in stem-cell maintenance. The study by Hirsch et al.[46] largely recapitulates pioneering findings by Li et al.[48] demonstrating that, conversely to chemotherapy treatment, the epidermal growth factor receptor [EGFR]/HER2 pathway inhibitor lapatinib decreased the subpopulation of chemotherapy-resistant breast cancer initiating cells. Of note, activation of AMPK (and downstream inhibition of anabolic metabolism including de novo fatty acid biogenesis) emerges as the common molecular target when comparing well-defined mechanisms of action through which both metformin and lapatinib exert their anti-cancer effects.[49] In this regard, we recently reported that exogenous supplementation with metformin synergistically interacted with lapatinib in HER2-positive breast cancer models that, upon upregulation of the AMPK regulator S6K1, have acquired auto-resistance to lapatinib.[28,30]

Regardless the relevance of metformin doses toward metformin's molecular target on either breast cancer cell compartment (IGF-1/IGF1-R1, AMPK/mTOR or both—a crucial issue that certainly merits to be addressed in future studies),—these findings strongly suggest that, in

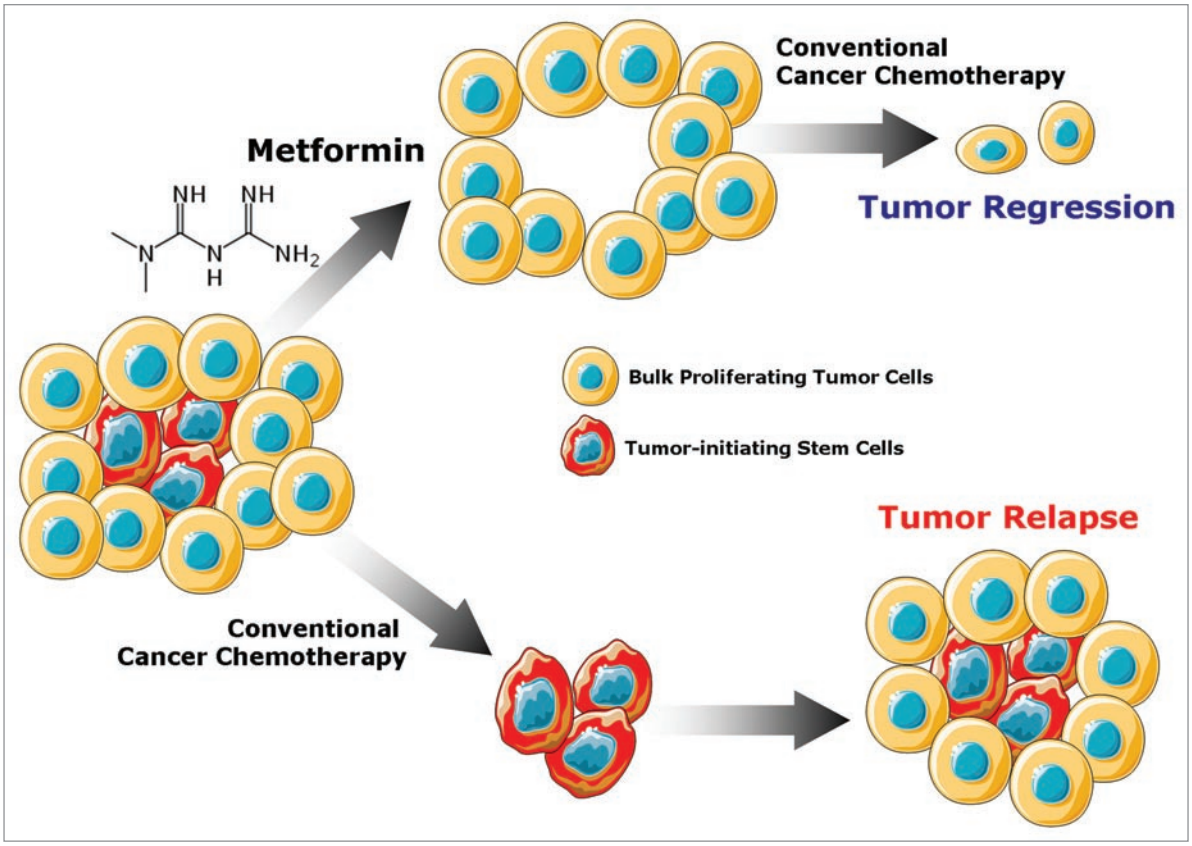

Figure 2. Metformin: A new drug to kill the "dandelion root" beneath the "cancer soil". When using conventional chemotherapy, the number of tumor cells decreases but the proportion of tumor-initiating stem cells is higher than before treatment, thus indicating that cytotoxics efficiently kill tumor cells whereas cancer stem cells, by their nature, are intrinsically resistant to the effects of anti-cancer drugs thereby allowing tumor regrowth. Analogous to the propensity of dandelion roots to regenerate weeds, regrowth of tumors from an intrinsically chemotherapy-resistant subpopulation has been termed the "dandelion hypothesis". When used in combination with traditional cytotoxic chemotherapy, the biguanide metformin doesn't just kill bulk proliferating tumor cells, but appears to be able to target the cancer-initiating stem cells from which tumor cells develop, thus preventing relapse of cancer disease. Consistent with the dandelion hypothesis, in which both dividing daughter cells and tumorigenic cancer cells must be targeted to prevent relapse, metformin together with conventional therapy would lead to a high pathological complete response rates in human malignancies.

combination with conventional therapy, metformin co-treatment may provide a successful therapeutic strategy to prevent cancer recurrence and improve long-term survival (**Fig. 2**). Perhaps, the unexpected ability of metformin treatment to attack just *the root of the dandelion* may largely explain the ability of standard clinical doses of metformin to significantly enhance the rate of pathological complete responses (pCR) in diabetic breast cancer patients receiving metformin and neoadjuvant chemotherapy.[41] To definitely test this hypothesis, disease-free survival (DFS) may be the best reflection (primary end point) of metformin activity against breast cancer stem cells—but this will require long studies and large patient numbers—with secondary end point of serially measuring the breast cancer stem cells in preclinical models in vitro. Alternatively, and given that: (a) breast cancer patients with HER2$^+$ disease who experience a pCR to neoadjuvant chemotherapy experience a better DFS with long-term follow-up;[50] (b) the HER2 pathway plays an important role in the maintenance of breast cancer stem cells;[51] (c) stem cells of HER2-positive breast carcinomas express the highest HER2 levels and are sensitive to the anti-HER2 monoclonal antibody trastuzumab,[52] and (d) HER2-positive pre-clinical models are differentially sensitive to the anti-tumor activity of metformin,[11,12,23,24] crucial evidence of the clinical efficacy of metformin can be obtained from small "proof-of-principle" studies with neoadjuvant regimens including neoadjuvant chemotherapy (to target bulk tumor cells) and trastuzumab with metformin (both targeting tumor-initiating cells) in women diagnosed with HER2-positive primary breast cancer.[53]

Metformin and Breast Cancer: A Hormetic Corollary

Even in the best-case molecular scenario for achieving metformin-related anti-breast cancer clinical benefits, we should acknowledge that, given the remarkably heterogenicity of breast cancer disease, it seems likely that the characteristics of metformin-sensitive bulk proliferating tumor cells and slow/non-dividing breast cancer-initiating stem cells may evolve under the selection pressure of chronic metformin treatments. Recent work by Anisimov et al.[54] in female SHR mice

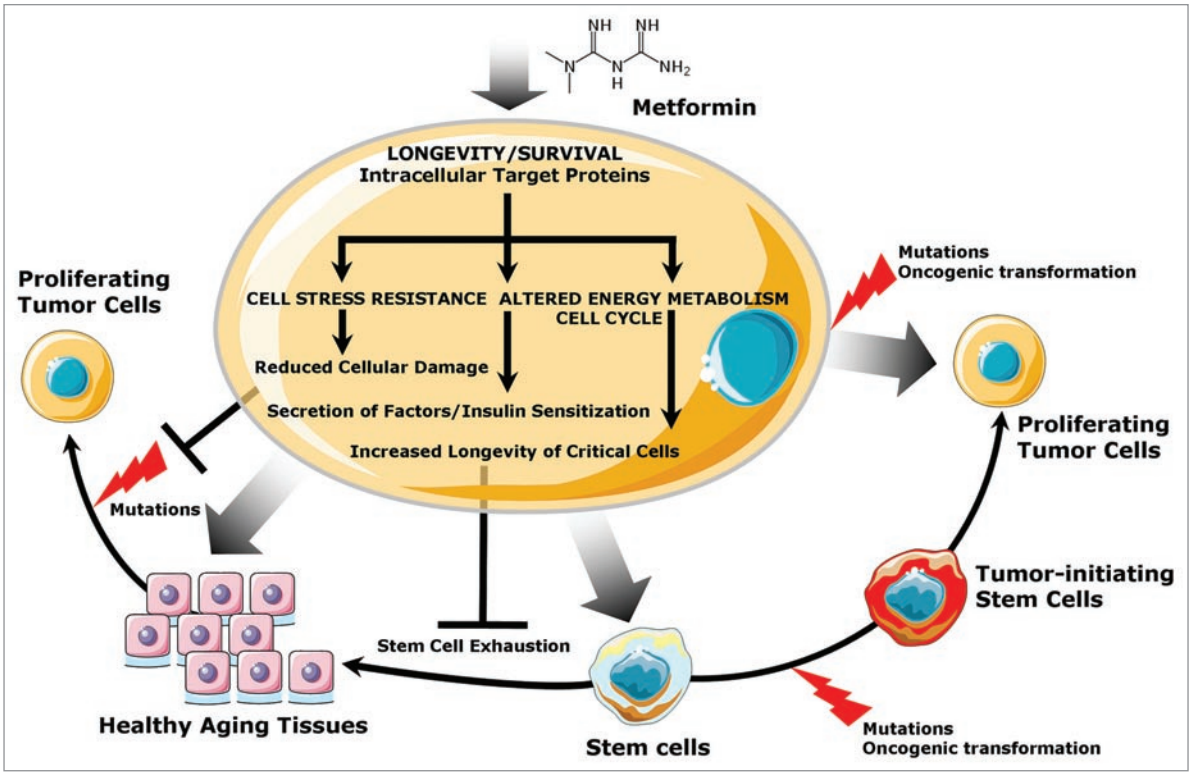

Figure 3. Anti-aging metformin: From cancer protection to hormesis danger. Use of microarrays biomarkers has identified metformin as a promising candidate caloric restriction (CR) mimetic. The major cellular processes targeted by the CR-mimicking effects of metformin are apoptosis and cell survival, differentiation and cell proliferation. Therefore, metformin may rapidly and strongly affect the molecular biology of mitotically competent tissues (which are prone to tumor formation) in a manner consistent with repair and elimination of damage through enhanced apoptosis while producing fewer rapid effects (in a manner consistent with lower levels of apoptosis and enhanced cell survival) in postmitotic tissues. However, the fact that metformin can stimulate adaptive responses in mitotically competent tissues could lead to counterintuitive detrimental effects (i.e., metformin-induced cell protection might exceed metformin-induced stress challenge). If metformin significantly enhances intrinsic capacity of mitotically competent cells to self-maintenance and repair (hormesis), metformin-promoted highly adaptive phenotypes (at the cellular level) may be positively selected (at the organismal level) to efficiently sustain fitness of transformed cells.

has revealed that, unlike in cancer-prone transgenic mice carrying the HER2 oncogene,[11,12] metformin feeding—at concentrations that approximately the clinical situation—unexpectedly fails to decrease the incidence of malignant tumors including mammary carcinomas while extending lifespan, thus suggesting some pleiotropic effects related to long-term treatments with metformin. By acting as a caloric restriction mimetic (CRM), it is expected that chronic administration of low levels of metformin can stimulate cell intrinsic capacity for self-maintenance and repair (hormesis by definition),[55] thus promoting beneficial anti-cancer/anti-aging effects. However, metformin-induced highly-adaptive "hormetic phenotypes" (at the cellular level) can be positively selected with harmful consequences (at the organismal level) if they efficiently sustain fitness of oncogenic transformed cells (**Fig. 3**).[56] Administration of metformin results in changes that closely parallel the metabolic and gene expression patterns of CR animals.[10,57] Consequently, metformin mimicked CR can elicit a protective survival response that promotes longevity and healthy aging. However, like DNA damage, hormetic responses to metformin-induced metabolic stress could trigger also development of cancer depending on the amount, location and the type of cell sustaining the damage.[58] Therefore, one cannot rule the possibility that interfering with glucose/insulin metabolism in a normal adult on a normal diet will never be completely safe. The ability of metformin to trigger anticancer responses by suppressing crucial metabolic axis while concurrently boosting defenses that maintain cell integrity with hormesis danger certainly merits to be examined in detail when moving ahead with planned chemopreventive, neoadjuvant and adjuvant metformin-based breast cancer trials.

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