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REVIEW

Glioma Stem Cells: Markers, Hallmarks and Therapeutic Targeting by Metformin

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Abstract Malignant gliomas are among the deadliest primary brain tumors. Despite multimodal therapy and advances in chemotherapy, imaging, surgical and radiation techniques, these tumors remain virtually incurable. Glioma stem cells may be responsible for resistance to traditional therapies and tumor recurrence. Therefore, elimination of glioma stem cells may be crucial for achieving therapeutic efficacy. Metformin, a small molecule drug widely used in the therapy of type 2 diabetes, has shown significant anti-tumor effects in patients with breast cancer and prostate cancer. Recent preclinical data suggest that metformin also has therapeutic effects against glioma. Here we review the markers and hallmarks of glioma stem cells, and the molecular mechanisms involved in therapeutic targeting of glioma stem cells by metformin.

Keywords Cancer stem cells · Glioma · Glioma stem cells · Metformin · Therapeutic targeting

Introduction

Glioma

The prognosis for patients with glioma is often very poor; this type of brain tumor remains one of the most lethal forms of human cancer [1–3]. Of the 77,000 patients diagnosed each year with glioblastoma (grade IV glioma) in the United States and Europe, less than 3 % will survive more than 5 years [4]. For patients with recurrent glioblastoma, survival time is typically measured in months [1, 2]. Although glioblastomas rarely spread outside the central nervous system (CNS), they are highly invasive and often infiltrate vital structures in the brain, which precludes curative surgical resection. Treatment failure is also attributable to ineffective delivery of chemotherapeutic agents across the blood–brain barrier (BBB), and associated dose-limiting systemic toxicities [5–8]. Traditional therapies, including radiation and chemotherapy, offer only modest benefits and remain essentially palliative. For these reasons, achieving a cure remains only a distant hope for patients with glioblastoma.

Glioma Stem Cells

An important progress in tumor biology has been the identification of a key population of tumor cells with stem cell properties, referred to as cancer stem cells (CSCs) [9–12]. These stem-cell-like cancer cells make up just a small fraction of the malignant cells in leukemia and many solid tumors, but there is increasing evidence that they are responsible for tumor initiation, propagation of the disease, resistance to current therapies and tumor recurrence [13–16]. Key properties that distinguish CSCs from the rest of the tumor cells include their ability to a) self-renew, b) differentiate into heterogeneous types of tumor cells, and c) sustain tumor growth in vivo. From a clinical point of view, a key characteristic of CSCs is their resistance to chemo- and radiotherapy [17–19], which may explain the limited efficacy of conventional therapeutic approaches that target the bulk of neoplastic cells, but may allow the CSCs to survive and regenerate the tumor.

Gliomas were among the first solid tumor type in which the existence of CSCs was experimentally demonstrated about a

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decade ago [20, 21]. Extensive experimental validation of glioma stem cells (GSCs) in various preclinical models has put gliomas at the forefront of cancer stem cell research, and provided an impetus to understanding of crucial cellular and molecular mechanisms of CSCs. GSCs have distinct markers (e.g. CD133, nestin) and molecular profiles that include ligands, receptors, intracellular signaling molecules, microRNAs, as well as transcription factors and chromatin-modifying proteins (Table 1) (for an extensive recent review see [13]). However, there is accumulating evidence that the intrinsic properties of GSCs are regulated by specific signals from the extracellular microenvironments – the so called niche (s) - in which these cells are located (Table 2). Such niches play an essential role in the maintenance of the undifferentiated stem cell-state of GSCs and their homeostasis. GSCs not only exploit preexisting microenvironments, but are involved in actively shaping these niches through an intricate crosstalk with various tissue components, both proximal and distal from the tumor, thus participating in a complex bidirectional crosstalk (Table 2). If cancer stem cells are crucial for the initiation, maintenance and recurrence of glioblastoma, then treatments designed to kill these cells may mean a paradigm shift in glioma therapy that could prove more effective than current therapies.

Therapeutic Approaches for Glioma

There is a huge volume of published work addressing various therapeutic approaches for glioma (>30,000 entries for 'glioma' and 'therapy' in the PubMed database). However, as mentioned above, a curative or highly effective therapy for glioma has proven elusive. Figure 1 gives a brief (non-comprehensive) summary of various therapeutic approaches for glioma, used in the clinical setting, as well as those that are in pre-clinical or experimental phase. Some of the major obstacles to successful therapy are also listed (Fig. 1). Identification of glioma stem cells, their cellular and molecular characterization, and research on glioma stem cell niches, may hold promise for developing therapies targeting specifically the glioma stem cell population, which may put glioma therapy into a new framework. Multiple potential strategies and therapeutic targets have been identified in glioma stem cells, which include: ***1) Direct glioma stem cell targeting strategies*****:** a) overcoming resistance to standard treatment, b) blocking function, c) inducing glioma stem cell differentiation; ***2) Indirect glioma stem cell targeting strategies*****:** a) targeting of perivascular niche, b) targeting of hypoxic niche, c) targeting of immune niche (for reviews see [13–15, 22, 23]).

There is emerging evidence that metformin (Fig. 2), in addition to being a first-line drug in the treatment of type 2 diabetes, and a drug used in the treatment of polycystic ovary syndrome, has shown significant promise in cancer prevention and therapy [24–28]. The anti-cancer effect of metformin occurs via multiple mechanisms, among which eradication of cancer stem cells is perhaps the most remarkable (summarized in Table 3). Below we discuss the mechanisms by which metformin targets glioma stem cells, and we also give examples of cancers in which metformin showed robust anti-cancer effects in clinical trials (e.g. breast cancer, prostate cancer).

Metformin

Metformin is the most widely used drug for treating patients with type 2 diabetes (prescribed to approx. 120 million patients with type 2 diabetes/year) (Fig. 2) [26, 29]. Its glucose-lowering effect is a consequence of reduced hepatic glucose production and increased glucose utilization, thus metformin is antihyperglycemic (not hypoglycemic) as it does not stimulate insulin release from the pancreas and generally does not cause hypoglycemia, even in large doses [30]. The history of metformin dates back to the use of *Galega officinalis* (goat's rue, French lilac, Italian fitch; a plant rich in guanidine) as a botanic medicine for the treatment of polyuria in medieval Europe. *G. officinalis* has been a long-recognized for its antihyperglycemic properties that led to the synthesis of the biguanide compound metformin (PubChem, [31]).

Targeting of Glioma and Glioma Stem Cells by Metformin

Metformin Causes Cell Cycle Arrest and Mitochondria-Dependent Apoptosis of Glioma Cells

Isakovic et al. [32] reported a dual antiglioma effect of metformin in vitro using rat C6 and human U251 glioma cell lines. In low-density cultures of the C6 rat glioma cell line, metformin blocked cell cycle progression in the G0/G1 phase without inducing significant cell death. In confluent C6 cultures, metformin caused induction of caspase-dependent apoptosis that was associated with activation of c-Jun N-terminal kinase (JNK), mitochondrial depolarization and oxidative stress. Apoptosis induced by metformin was prevented by cyclosporine A that blocks mitochondrial permeability transition, and N-acetylcysteine that blocks oxygen free radical production. Inhibition of JNK activation by SP600125 or glycolysis by sodium fluoride or iodoacetate provided partial protection from metformin-induced apoptosis. The antiglioma effect of metformin was reduced by compound C, an inhibitor of AMP-activated protein kinase (AMPK), a molecular hub for cellular metabolic control [33]. The AMPK agonist 5-aminoimidazole-4-carboxamide 1-beta-D-ribofuranoside (AICAR) mimicked the antiglioma effect of metformin.

Table 1 Glioma stem cell markers and molecules involved in GSC maintenance [13, 15]

GSC markers	Ligands	Receptors	Intracellular molecules	Transcription factors, chromatin-modifying proteins
• ABCG2 • ALDH1A1 • Bmi-1 • CD44 • CD90 • CD133 • EGFR • HIF-1α • HIF-2-α • Integrin α6 • Musashi • Nanog • Nestin • Olig2 • Sox2 • SSEA1	• BMP4 • PDGF-B • SHH • TGFβ • WNT	• Notch • C-Met • CXCR4 • EGFR; • EGFRvIII • IL6Rα • L1CAM • PDGFRβ • VEGFR2	• A20 • Akt • BMX • IGFBP-2 • miR-7 • miR-34a • miR-128 • miR-302–367 cluster • miR-326 • miR451 • NOS-2 • Rac	• Bmi-1 • c-myc • STAT3

Abbreviations

GSC markers

ABCG2, ATP-binding cassette subfamily G member 2, the homodimerized form is involved in molecular transport, its expression is upregulated under low-oxygen conditions; **ALDH1A1**, Aldehyde dehydrogenase 1 family, member A1; **Bmi-1**, B cell-specific Moloney murine leukemia virus integration site 1, also known as polycomb complex protein Bmi-1; **CD44**, Cell-surface glycoprotein involved in cell–cell interactions, cell adhesion and migration, receptor for hyaluronic acid; **CD90** (Thy-1) N-glycosylated, glycophosphatidylinositol (GPI)-anchored cell surface protein, immunoglobulin superfamily member; **CD133** (AC133, prominin 1), Pentaspan transmembrane glycoprotein; **EGFR**, Epidermal growth factor receptor (ErbB-1; HER1 in human); **HIF-1α**, Hypoxia-inducible factor 1α, transcription factor that responds to changes in available oxygen in the cellular environment; **Integrin α6**, Receptor for extracellular matrix molecules (e.g. laminin); **Musashi**, RNA-binding protein that regulates the expression of target mRNAs; **Nanog**, Transcription regulator involved in inner cell mass and embryonic stem cell proliferation and self-renewal; **Nestin**, Intermediate filament protein; **Olig2**, Oligodendrocyte lineage transcription factor 2, required for oligodendrocyte and motor neuron specification in the spinal cord, as well as for the development of somatic motor neurons in the hindbrain; **Sox2**, SRY (sex determining region Y)-box 2, transcription factor critical for early embryogenesis and embryonic stem cell pluripotency; **SSEA1**, Stage-specific embryonic antigen 1

Ligands

BMP4, Bone morphogenetic protein 4, induces cartilage and bone formation; **PDGF-B**, Platelet-derived growth factor subunit B, a potent mitogen for cells of mesenchymal origin; **SHH**, Sonic hedgehog, it binds to the patched (PTC) receptor, which functions in association with smoothened (SMO), to activate the transcription of target genes.; **TGFβ**, Transforming growth factor β, multifunctional protein that controls proliferation, differentiation and other functions in many cell types; **WNT**, a family of cysteine-rich glycoproteins, critical in establishing the polarity of insect and vertebrate limbs, promote the proliferation of stem cells

Receptors

Notch, Receptor for Delta, Jagged, or Serrate, participants in juxtacrine interactions, ligand binding causes Notch to undergo a conformational change that enables a part of its cytoplasmic domain to be cut off by the Presenilin-1 protease, the cleaved portion enters the nucleus and binds to a dormant transcription factor of the CST family; **c-Met**, Proto-oncogene that encodes the receptor for hepatocyte growth factor/scatter factor, which has a tyrosine-protein kinase activity, functions in cell proliferation, scattering, morphogenesis and survival; **CXCR4**, Receptor for the C-X-C chemokine CXCL12/SDF-1, involved in hematopoiesis, cardiac ventricular septum formation, chemotactic activity of lymphocytes, essential role in vascularization of the gastrointestinal tract; **EGFR**, Epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian), receptor for EGF and other members of the EGF family (e.g. TGF-alpha, amphiregulin, betacellulin, heparin-binding EGF-like growth factor, GP30 and vaccinia virus growth factor), involved in the control of cell growth and differentiation; **EGFRvIII**, EGF receptor variant III, A truncated and constitutively active form of EGF receptor, major determinant of tumor growth and progression in glioblastoma multiforme; **IL6Rα**, Interleukin 6 receptor alpha (also known as CD126) is a type I cytokine receptor, involved in cell growth, differentiation and immune function; **Integrin α6**, Receptor for laminin, critical structural role in the hemidesmosome; **L1CAM**, L1 cell adhesion molecule, important role in the development of the nervous system (neuron-neuron adhesion, neurite fasciculation, outgrowth of neurites); **PDGFRβ**, Platelet-derived growth factor receptor beta; specifically binds PDGFB and PDGFD, has tyrosine-protein kinase activity; **VGFR2**, Vascular endothelial growth factor receptor 2 (Flk-1/KDR), a type III receptor tyrosine kinase, involved in angiogenesis

Intracellular molecules

A20 (TNFAIP3), Tumor necrosis factor, alpha-induced protein 3, essential component of a ubiquitin-editing protein complex; **Akt (protein kinase B, PKB)**, a serine/threonine-specific protein kinase that plays a key role in multiple cellular processes (glucose metabolism, apoptosis, cell proliferation, transcription and cell migration); **BMX**, A non-receptor tyrosine kinase, required for IL-6 induced differentiation; **IGFBP-2**, Insulin-like growth factor binding protein 2, prolongs the half-life of the IGFs; **miR**, microRNA; **NOS-2**, Nitric oxide synthase; **Rac**, Member of the Rho family of GTPases

Transcription factors, **chromatin-modifying enzymes**

BMI-1, Component of the polycomb group (PcG) multiprotein PRC1 complex, required for maintaining the transcriptionally repressive state of many genes, including Hox genes; **c-myc**, v-myc myelocytomatosis viral oncogene homolog (avian), participates in the regulation of gene transcription

STAT3, Signal transducer and activator of transcription 3, transcription factor that binds to IL-6 responsive elements identified in the promoters of various acute-phase protein genes, mediates the expression of a variety of genes in response to cell stimuli, plays a key role in cell growth and apoptosis

Table 2 Hallmarks of glioma stem cells and their niche. A bidirectional crosstalk exists between GSCs and their niche [13, 22, 50–52]

GSC		Niche
Expression of stemness genes		
Self-renewal		
Replicative immortality	⇐	
Genomic instability		
Tumor regeneration		
Resistance to radiation and chemotherapy		
Resisting cell death		
Promotion of angiogenesis		
Recruitment of endothelial progenitor cells	⇒	
Differentiation into endothelial-like cells		
Promotion of invasion		
Avoiding immune destruction		
Promoting immunosuppression		
Deregulated cellular energetics		
Shaping of and crosstalk with the niche		

Perivascular and non-perivascular niches
Hypoxia
Glycolytic phenotype
Increased lactate production
Maintenance of GSCs
Tumor-associated macrophages (TAMs; immunosuppressive M2 phenotype)
Induction of Tregs

Similar effects of metformin were observed in the U251 human glioma cell line. Of note, rat primary astrocytes were resistant to the antiproliferative and proapoptotic action of metformin. Thus, metformin causes cell cycle arrest and apoptosis of glioma cells, but not of normal astrocytes.

In an independent study, Liu and colleagues reported that AMPK is abundantly expressed in high-grade gliomas and that metformin and AICAR (AMPK activators) suppressed glioma cell proliferation through unique AMPK-independent mechanisms [34]. Metformin directly inhibited mTOR by enhancing association of PRAS40 with RAPTOR, whereas AICAR blocked the cell cycle through proteasomal degradation of the G2M phosphatase cdc25c. It should be noted that in this study human glioma cells (T98G and U87EGFRvIII) but

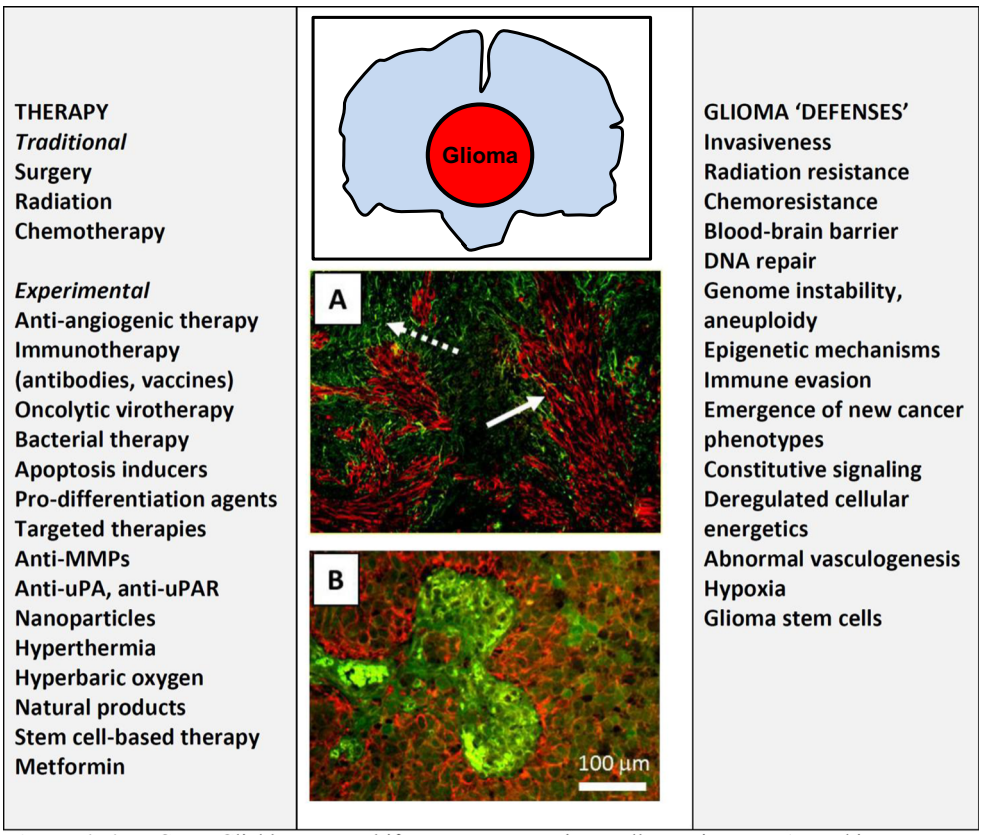

Fig. 1 Traditional and experimental therapies for glioma and possible mechanisms of resistance to therapy. **a**, Interdigitation of invasive human nestin+U251 glioma cells (*red*, *solid arrow*) and mouse nestin+ host mouse cells (*green*, *dotted arrow*). **b**, Host (*rat*) nestin+ glomerular microvascular proliferation (*green*) typical of human GBM pathology recapitulated in a human glioma xenograft (*red*) in nude rat brain. Such glomerulus-like microvasculature may not be fully functional and may result in hypoxia, a hallmark of glioma (panels **a**, **b** adopted from [49])

Abbreviations GBM, Glioblastoma multiforme; MMP, Matrix metalloproteinase; uPA, Urokinase plasmigonen activator; uPAR, Urokinase plasmigonen activator receptor

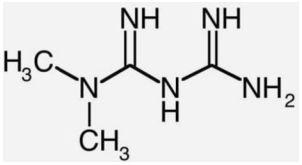

Fig. 2 Metformin [3-(diaminomethylidene)-1,1-dimethylguanidine; molecular weight 129.2)]

not glioma stem cells were used, thus it is still an open question whether these findings apply also to glioma stem cells.

Metformin Selectively Affects Human Tumor-Initiating Cell (Glioma Stem Cell) Viability via Inhibition of Akt

Würth et al. [35] used tumor-initiating cells (TICs) isolated from patients with glioblastoma multiforme. They demonstrated that these tumor-initiating cells (glioma stem cells) could generate glioma in non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice after orthotopic implantation of 100, 1,000, or 10,000 tumor-initiating cells (tumor take was ~20, ~40, and 100 % for the indicated cell doses, respectively). The authors reported that metformin treatment in vitro reduced the proliferation rate of tumor-initiating cells isolated from glioblastoma patients. Metformin also inhibited spherogenesis by tumor-initiating cells, suggesting a direct effect on the self-renewal mechanisms in TICs. Of note, flow cytometry analysis of the antiproliferative effects of metformin on $CD133^+$ subpopulation (CD133 is a marker of glioma and other types of cancer stem cells), a higher reduction of proliferation was observed when compared to $CD133^-$ cells. This suggested a degree of selectivity in the action of metformin against glioma stem cells. In line with this, differentiation of glioblastoma cells strongly reduced the sensitivity to metformin treatment. The effect of metformin on tumor initiating cell-enriched cultures was associated with a robust inhibition of Akt-dependent cell survival pathway, while this signaling pathway was not affected in differentiated glioma cells. The specificity of the antiproliferative effect of metformin toward glioblastoma tumor-initiating cells was confirmed by lack of significant inhibition of proliferation of normal human stem cells (umbilical cord-derived mesenchymal stem cells) after in vitro exposure to metformin. These data suggest that metformin exerts antiproliferative activity on glioblastoma cells, showing a higher specificity toward tumor-initiating cells. Inhibition of Akt pathway may be one of the molecular mechanisms underlying the antiglioma effect of metformin.

Table 3 Possible mechanisms of anti-cancer activity of metformin [33, 53–55]

Anti-cancer actions of metformin	• LKB1/AMPK pathway activation • Cell cycle arrest and/or apoptosis • Inhibition of protein synthesis • Reduction of circulating insulin levels • Inhibition of unfolded protein response • Immune system activation • Inhibition of EMT (inhibition of invasiveness) • Increasing the expression of miRNAs antagonizing cancer progression • Lowering the threshold of tumor cell senescence • Enhancing the therapeutic effect of chemotherapeutic drugs • Eradication of cancer stem cells

Metformin Therapy Eliminates Glioma-Initiating Cells and Extends the Life of Orthotopic Glioma Xenograft-Bearing Mice

Elimination of the cancer stem/initiating cell population is considered to be a key factor in achieving effective therapy and long-term survival of patients with various cancers, including patients with glioblastoma. Sunayama et al. [36] demonstrated that activation of the forkhead box O3 (FOXO3) transcription factor is sufficient to induce differentiation of glioma-initiating cells with stem-like properties (glioma stem cells) and inhibit their tumor-initiating potential. Subsequently, in an elegant study Sato and colleagues [37] identified metformin as a therapeutic activator of FOXO3. Activation of FOXO3 by metformin promoted the differentiation of stem-like glioma-initiating cells into non-tumorigenic cells. The metformin-induced promotion of FOXO3 activation and glioma stem cell differentiation occurred via activation of AMP-activated protein kinase (AMPK), which was sensitive to extracellular glucose concentration. The latter finding suggested a novel and direct link between glucose metabolism and glioma stem cells. A key finding was that transient, systemic administration of metformin resulted in depletion of the self-renewing, tumor-initiating cell population within established intracranial tumor xenografts in BALB/cAJcl-*nu*/*nu* mice, and metformin inhibited tumor formation by stem-like glioma-initiating cells in the brain, and provided a substantial survival benefit of glioma-bearing mice. These findings demonstrate that therapeutic targeting of glioma-initiating cells via the AMPK-FOXO3 axis may be an effective strategy against glioblastoma. Considering the fact that metformin is already used safely in the clinic and that it efficiently penetrates the blood–brain barrier and accumulates in the brain parenchyma [38], the findings of Sato et al. [37] suggest that metformin is a strong candidate for clinical use as a cancer stem cell-targeting drug against glioblastoma. Based on these data metformin appears to be the most clinically relevant

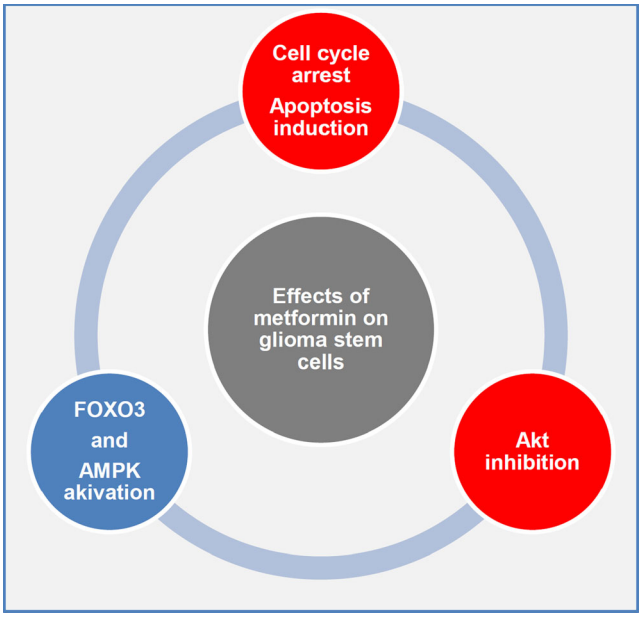

Fig. 3 Schematic summary of the effects of metformin on glioma stem cells

drug reported for specific targeting of glioma stem/initiating cells. A schematic summary of the recently demonstrated mechanisms by which metformin exerts its effects on glioma stem cells is shown in Fig. 3.

Clinical Evidence for Therapeutic Effect of Metformin in Patients With Breast Cancer and Prostate Cancer

Breast Cancer

Population studies have suggested that metformin use results in decreased incidence of cancer and cancer-related mortality in patients with type 2 diabetes [39, 40]. Jiralerspong et al. [41] carried out a study aimed at determining whether metformin use was associated with a change in pathologic complete response (pCR) rates in diabetic patients with breast cancer receiving neoadjuvant chemotherapy. The study involved 2,529 patients with early-stage breast cancer (68 patients also had diabetes and were taking metformin, 87 had diabetes but were not taking metformin, and 2,374 patients were nondiabetic). The authors reported that the rate of pCR was 24 % in the metformin group, 8.0 % in the nonmetformin group, and 16 % in the nondiabetic breast cancer group (P=0.02). Of note, metformin use was independently predictive of pCR (odds ratio, 2.95; P=0.04) after adjustment for diabetes, body mass index, age, cancer stage, grade, receptor status, and use of taxane neoadjuvant. In a subsequent study, Chlebowski et al. [42] assessed the associations among diabetes, metformin use, and breast cancer in postmenopausal women who were participating in Women's Health Initiative clinical trials. A total of 68,019 postmenopausal women, which included 3,401 with diabetes at study entry, were observed over a mean of 11.8 years during which 3,273 invasive breast cancer cases were diagnosed. Compared with that in women without diabetes, breast cancer incidence in women with diabetes differed by diabetes medication type (P=0.04). Women with diabetes who received medications other than metformin had a slightly higher incidence of breast cancer (hazard ratio [HR], 1.16; 95 % CI, 0.93 to 1.45), and women with diabetes who were given metformin had lower breast cancer incidence (HR, 0.75; 95 % CI, 0.57 to 0.99). Such association was observed for breast cancers positive for both estrogen receptor and progesterone receptor, as well as those that were negative for expression of human epidermal growth factor receptor 2. The authors concluded that metformin use in postmenopausal women with diabetes was associated with lower incidence of invasive breast cancer. These data may have an impact on future studies aimed at evaluating metformin use in breast cancer management and prevention.

In vitro and preclinical studies Hirsch et al. [43] demonstrated that metformin selectively kills cancer stem cells in four genetically different types of breast cancer (MCF10A ER-Src, MCF7, SKBR3, MDA-MB-468). The combination of metformin and doxorubicin (a well-defined chemotherapeutic drug) killed both breast cancer stem cells and non–stem cancer cells in culture, and reduced tumor mass and prolonged remission much more effectively than either drug alone in a xenograft *nu/nu* mouse model. The authors pointed out that their data provide a rationale for why the combination of metformin and chemotherapeutic drugs might improve treatment of patients with breast cancers, and they hypothesized that such a therapeutic strategy may be applicable to other types of cancer as well. In a more recent study Hirsch et al. showed that metformin inhibits the inflammatory response associated with cellular transformation and cancer stem cell growth [44]. Using a Src-inducible model of cellular transformation the authors demonstrated that metformin inhibited the activation of the inflammatory transcription factor NF-κB and strongly delayed cellular transformation. The inhibition of transformation did not occur when metformin was added after the initial inflammatory stimulus. Metformin preferentially inhibited the translocation of NF-κB into the nucleus and phosphorylation of STAT3 in cancer stem cells when compared with non-stem cancer cells. The data strongly suggest that a decreased function of inflammatory feedback loop involving Lin28B and IL1β (downstream targets of NF-κB)

may underlie the inhibition of tumor growth and prolonged remission in mice with breast cancer xenografts treated with a combination of metformin and doxorubicin. Based on these data and because metformin alters energy metabolism in diabetics, the authors suggested that metformin may block the metabolic stress response that stimulates the inflammatory pathway associated with a wide variety of cancers (e.g. prostate cancer, melanoma).

Prostate Cancer

Margel et al. [45] evaluated the benefits of metformin use in patients with prostate cancer (PC) who were taking metformin for their type 2 diabetes. The investigators used a population-based retrospective cohort design for their study, and obtained data from Ontario, Canada health care administrative databases. The study focused on a cohort of men older than 66 years of age with incident diabetes who subsequently developed PC (total 3,837 patients, median age at PC diagnosis 75 years, median follow-up 4.64 years). The authors reported that the cumulative duration of metformin treatment after PC diagnosis was associated with a significantly decreased risk of PC-specific and all-cause mortality in a dose-dependent fashion. The adjusted hazard ratio for PC-specific mortality was 0.76 for each additional 6 months of metformin use. The association with all-cause mortality was also significant but declined over time from a hazard ratio of 0.76 in the first 6 months to 0.93 between 24 and 30 months. Of note, there was no relationship between cumulative use of other antidiabetic drugs (sulfonylurea, thiazolidinedione, insulin) and either outcome. The clinical implications of these findings are three-fold: 1) metformin may be considered as first-line therapy for patients with PC and diabetes, not only for diabetes control but possibly to improve cancer prognosis; 2) because metformin was associated with benefit regardless of other cancer treatments, metformin may further improve patient survival as an adjunct therapy; 3) metformin may be effective for secondary prevention because it is safe, well-tolerated, and inexpensive. In summary, increased cumulative duration of metformin exposure after PC diagnosis was found to be associated with decreased PC-specific and all-cause mortality among diabetic men.

Clinical Trials

Quinn et al. published a featured review on ongoing and upcoming clinical trials on the use of metformin for cancer treatment and prevention [24]. Numerous types of cancer interventions (breast cancer, endometrial cancer, pancreas cancer, prostate cancer, colorectal cancer, malignant melanoma, hematologic malignancy, lung cancer, head and neck cancer, ovarian cancer, glioblastoma, and other cancers) have been included in clinical trials testing the effect of metformin in combination with various therapeutics. The target doses of metformin ranged from 500 to 2,550 mg/day, and the planned duration of metformin treatment was a few weeks to several months (with one trial in breast cancer patients planned to last 5 years).

The authors also summarized the ongoing and upcoming clinical trials for cancer chemoprevention with metformin. The study groups included: non-diabetic patients at high risk for cardiovascular disease, obese patients at elevated risk for breast cancer, obese breast cancer survivors, obese post-menopausal patients at elevated risk for endometrial cancer, patients with a recent history of colorectal adenoma, and patients with Barrett's esophagus.

On the US Government's Clinical Trials website (www.clinicaltrials.gov) there are currently a total of 200 studies listed under the search terms 'cancer metformin' (accession date, May 15, 2014). Using the search terms 'glioma metformin', currently one study can be found, which is at MD Anderson Cancer Center in Texas. Trial identifier: NCT01430351; phase I; purpose: 'The goal of this clinical research study is to find the highest tolerable dose of temozolomide in combination with memantine, mefloquine, and/or metformin that can be given to patients with glioblastoma who have already been given radiation and chemotherapy in combination. The safety of these drug combinations will also be studied'; drugs to be tested: temozolomide, memantine, mefloquine, metformin; estimated enrollment: 144; start date: September 2011; estimated primary completion date: September 2015; primary outcome measures: maximum tolerated dose (MTD), progression free survival (PFS).

Conclusion

Development of effective therapy for glioma remains a significant challenge in oncology. However, advances in understanding the pathomechanisms of glioma, identification of glioma stem cells and of therapeutic targets in the glioma stem cell population gives us hope for advent of more effective glioma therapies. Metformin appears to be a highly promising agent in the therapy of various cancers, including breast cancer and prostate cancer. Metformin is taking a special place among small molecule drugs that have anticancer effects, especially because it targets cancer stem cells, it has been used for decades for therapy on type 2 diabetes with very few side-effects, and it is available at low cost. With recent studies showing that metformin has anti-glioma effects in vitro and in pre-clinical animal models of glioma, there is renewed hope

and impetus for further investigation of metformin as an adjunct therapy for glioma and other cancers.

Box 1. Outstanding questions
- Will metformin prove to be effective in the treatment of patients with glioma as in other types of cancer? Current and future clinical trials should answer this question.
- Which types and subtypes of glioma (and possibly other brain tumors) are the most promising targets for treatment using metformin?
- When, how long and at what doses should metformin be used in glioma therapy?
- What types of combination therapy with metformin could be clinically useful for glioma treatment? Are there any synergistic effects between metformin, other agents and/or stem cell-based targeted gene therapies [46]? Can metformin sensitize glioma cells to chemotherapy, radiation therapy and/or cycles of fasting [47]?
- Does metformin have epigenetic effects in glioma? If so, are there drugs acting on the epigenome of glioma that would have additive or synergistic effect with metformin [48]?
- Could metformin be used as part of a chemoprevention strategy to reduce the risk of developing glioma and other types of brain tumors?

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