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## FORUM REVIEW ARTICLE

# Glioblastomas: Hijacking Metabolism to Build a Flexible Shield for Therapy Resistance

Justine Bailleul<sup>1</sup> and Erina Vlashi<sup>1,2</sup>

### Abstract

**Significance:** Glioblastomas (GBMs) are among the most lethal tumors despite the almost exclusive localization to the brain. This is largely due to therapeutic resistance. Radiation and chemotherapy significantly increase the survival for GBM patients, however, GBMs always recur, and the median overall survival is just over a year. Proposed reasons for such intractable resistance to therapy are numerous and include tumor metabolism, in particular, the ability of tumor cells to reconfigure metabolic fluxes on demand (metabolic plasticity). Understanding how the hard-wired, oncogene-driven metabolic tendencies of GBMs intersect with flexible, context-induced metabolic rewiring promises to reveal novel approaches for combating therapy resistance.

**Recent Advances:** Personalized genome-scale metabolic flux models have recently provided evidence that metabolic flexibility promotes radiation resistance in cancer and identified tumor redox metabolism as a major predictor for resistance to radiation therapy (RT). It was demonstrated that radioresistant tumors, including GBM, reroute metabolic fluxes to boost the levels of reducing factors of the cell, thus enhancing clearance of reactive oxygen species that are generated during RT and promoting survival.

**Critical Issues:** The current body of knowledge from published studies strongly supports the notion that robust metabolic plasticity can act as a (flexible) shield against the cytotoxic effects of standard GBM therapies, thus driving therapy resistance. The limited understanding of the critical drivers of such metabolic plasticity hampers the rational design of effective combination therapies.

**Future Directions:** Identifying and targeting regulators of metabolic plasticity, rather than specific metabolic pathways, in combination with standard-of-care treatments have the potential to improve therapeutic outcomes in GBM. *Antioxid. Redox Signal.* 39, 957–979.

**Keywords:** glioblastoma, metabolism, plasticity, redox

### Introduction

**G**LIOLASTOMA, PREVIOUSLY KNOWN as glioblastoma multiforme (GBM), is a grade IV astrocytoma, the most common and most lethal primary brain tumor with a current 5-year survival rate of ~5% in the United States. Without prompt treatment, the survival of GBM patients is only about 3 months (Schapira, 2007). Maximal safe resection is the first attempt at controlling the tumor, followed by radiation therapy

(RT) given in 2 Gy fractions over 6 weeks for a total dose of 60 Gy. Ever since Walker et al. (1979, 1978) demonstrated in the late 1970s the effectiveness of postsurgical RT in significantly increasing the survival in GBM patients, RT has remained an indispensable treatment for GBM, extending the median survival to about 1 year (Stupp et al., 2009).

Despite extensive efforts to further improve survival with RT, only one alkylating agent, temozolomide (TMZ), has increased GBM median survival to ~15 months (Stupp et al.,

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2009; Stupp et al., 2005) in combination with RT. Therefore, current standard-of-care for treating GBM remains postsurgical RT with concurrent and adjuvant TMZ. Recently, tumor treating fields have shown some promise as an alternative strategy for treating GBM (Fabian et al., 2019), but this approach is yet to enter clinical practice.

GBM can be highly invasive, but metastasis outside the brain is extremely rare (Sullivan et al., 2014). RT can shrink or even cure most localized tumors, but GBM recurrence is almost universal, and often occurs within the irradiated field, pointing to a remarkable resistance to RT. Proposed reasons for such resistance are numerous, and include tumor metabolism. A comprehensive metabolic profiling of gliomas has revealed that distinct metabolic profiles associate with tumor grades in GBM (Chinnaiyan et al., 2012) pointing to a key role for tumor metabolism in GBM progression. GBMs are a heterogeneous group of tumors classified into four different subtypes, mesenchymal, classical, proneural, and neural, based on gene expression profiles (Brennan et al., 2013; Verhaak et al., 2010).

Another important classification is based on the mutational status of isocitrate dehydrogenase (IDH1/2), which occurs in ~10% of GBM patients (Louis et al., 2016). The implications of IDH mutations in metabolism and therapy response have been recently reviewed (Zhou and Wahl, 2019) and are therefore out of the scope of this review. The dynamic nature of GBM metabolism and its interactions with the tumor microenvironment (TME) have been recently reviewed by Morrow et al. (2021) and we have recently reviewed the extent of radiation effects on tumor metabolism in general, elsewhere (Read et al., 2022). In this study, we explore the role of tumor metabolism in protecting GBM from the cytotoxic effects of the current standard-of-care therapies and attempt to provide some insight into the potential of combining existing therapies with metabolic regulators to improve GBM outcomes.

Metabolic reprogramming is an emerging hallmark of cancer (Hanahan and Weinberg, 2011) and is largely driven by oncogenic mutations (Pavlova and Thompson, 2016). “Metabolic reprogramming” generally refers to sustained, long-term changes in the overall cellular metabolic state to support a specific function. In the case of tumors, during transformation, cancer cells reprogram their metabolism to sustain a rapid proliferation rate while surviving in a nutrient-poor TME. Over the last decade it has been appreciated that, once “reprogrammed,” tumor metabolism is not a rigid metabolic state. Rather, tumors exhibit remarkable metabolic plasticity or flexibility, generally defined as the capacity of a cell to reroute metabolic fluxes on-demand, based on its needs and nutrient availability. It is a dynamic process that allows cancer cells to adapt to an ever-changing TME or to therapy-induced stress.

Such metabolic changes are thought to be of a transient nature aimed at surviving a finite stress, for example, adapt to restrictions in the bioavailability of a nutrient or oxygen or respond to the cytotoxic effects of an anticancer therapy, that is, potentially lethal oxidative stress or DNA damage generated during RT, the backbone treatment for GBM. Some of the same metabolic pathways that generate antioxidants in cells (see: Glycolytic Branches: A Reservoir of Building Blocks and Antioxidant Defenses That Can Promote Therapy Resistance) are also the main producers of precursors for nucleotide synthesis, which would further support DNA re-

pair in radioresistant tumors following damage by oxidative stress. The metabolic redox changes that perpetuate oxidative stress induced by radiation in cells in general are thoughtfully reviewed by Spitz et al. (2004). In this study, we focus on studies that shed light into prosurvival metabolic reprogramming following radiation of cancer cells and the impact of specific oncogenic mutations, with emphasis on GBM.

### The Impact of Oncogenic Mutations on GBM Glucose Metabolism and Implications for Therapeutic Resistance

Based on extensive evidence, it is now largely accepted that most malignant cells enhance their glucose consumption to sustain the nutrient and bioenergetic needs of rapid proliferation, and GBM is no different. This is reflected in the “Warburg effect,” characterized by enhanced oxidation of glucose to lactate *via* glycolysis even when oxygen is plentiful (aerobic glycolysis) (DeBerardinis and Chandel, 2020; Warburg, 1924). In GBM, increased glucose uptake correlates with enhanced expression of glucose transporters (GLUTs), particularly GLUT1 (solute carrier family 2 member 1, *SLC2A1*) and GLUT3 (solute carrier family 2 member 3, *SLC2A3*) (Boado et al., 1994; Cosset et al., 2017; Flavahan et al., 2013), which are the predominant isoforms expressed in GBM and the normal brain.

The expression levels of all glycolytic genes are increased in GBM relative to low-grade gliomas (LGG) (Stanke et al., 2021), and a higher expression of certain glycolytic genes, including *SLC2A3*, hexokinase 2 (*HK2*), and the M2 isoform of pyruvate kinase (*PKM2*), is associated with poorer survival in GBM (Flavahan et al., 2013; Stanke et al., 2021). Moreover, higher lactate levels in the cerebrospinal fluid of GBM patients are associated with worse survival (Nakamizo et al., 2013). Together, these findings point to a supportive role for enhanced glycolysis in GBM progression.

The most common mutations in GBM are implicated in modifying glucose metabolism in a way that benefits tumor growth, as well as promotes therapy resistance. GBMs are characterized by activating mutations in the epidermal growth factor receptor (*EGFR*) gene, inactivation of phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*), or mutations in the *TP53* gene that result in loss of its tumor suppressor functions. Below, we summarize findings that shed light into the effects that these mutational events have on glucose metabolism. Where possible, we highlight GBM-specific studies, but in the lack thereof, we include discussions of other tumor models to glean insight into the potential consequences for GBM metabolism and response to therapy.

#### Alterations in EGFR-PTEN axis impact GBM glucose metabolism

EGFR pathway alterations significantly correlate with inferior prognosis in GBM (Brennan et al., 2013; Cancer Genome Atlas Research Network, 2008; Huang et al., 2009) and arise from gene amplification, protein overexpression, autocrine signaling loops, and/or mutations resulting in constitutive activation of EGFR, such as EGFR variant III (EGFR-vIII) (Huang et al., 2009). *EGFR* amplification and/or activating mutations are present in ~57% of GBM tumors (Brennan et al., 2013), whereas *PTEN* homozygous deletion occurs in ~36% (Cancer Genome Atlas Research Network, 2008), across all the different GBM subtypes (Verhaak et al.,

2010). PTEN antagonizes EGFR signaling by preventing the activation of AKT and downstream signaling, and therefore, loss of PTEN would be expected to enhance the metabolic consequences of hyperactive EGFR signaling. EGFR mutations and PTEN loss may co-occur, but no significant association between these genomic alterations has been identified (Yan et al., 2020).

EGFR signaling regulates glucose uptake. In other EGFR-mutated cancers, such as lung cancers, EGFR signaling regulates glucose consumption and expression of GLUTs (Kim et al., 2018; Makinoshima et al., 2014). In GBM, there is evidence that mutant EGFR-vIII upregulates the Myc-binding protein Delta Max, which activates Myc (Babic et al., 2013), subsequently increasing the expression of its target genes, including the glycolytic genes, *SLC2A1*, *SLC2A3* (GLUTs GLUT1/3), and *HK2* (Fig. 1). This enhances fluorodeoxyglucose (FDG) uptake *in vivo* (Babic et al., 2013) and induces a dependency on glycolysis in GBM tumors (Tateishi et al., 2016). Further supporting a link between EGFR signaling and glucose metabolism in GBM, a study by Mai et al. shows that inhibition of EGFR signaling *via* erlotinib decreases glucose consumption in a subset of human GBM tumors in mouse models of patient-derived xenografts. However, no specific *EGFR* mutation or other pathway alterations could predict this metabolic response to EGFR inhibition (Mai et al., 2017).

EGFR-PTEN axis regulates phosphoglycerate kinase 1. EGFR signaling can also promote glycolysis by activating phosphoglycerate kinase 1 (PGK1) (Li et al., 2016b) (Fig. 1). While PGK1 is normally autophosphorylated (on Y324), EGFR signaling leads to ERK-dependent phosphorylation of PGK1, although on a different residue (S203) (Li et al., 2016b) (Fig. 1). In addition to its function as a glycolytic enzyme, phosphorylated PGK1 can moonlight as a protein kinase (Lu and Hunter, 2018). It translocates to the mitochondria where it phosphorylates and activates pyruvate dehydrogenase kinase 1 (PDK1) (Li et al., 2016b). PDK1 inhibits pyruvate dehydrogenase complex (PDH), leading to a decrease in the production of acetyl-CoA from pyruvate and attenuation of the tricarboxylic acid (TCA) cycle (Golias et al., 2019) (Fig. 1). There is GBM-specific evidence that PTEN dephosphorylates (on Y324) and inactivates PGK1 (Qian et al., 2019) and that enhanced EGFR signaling dampens mitochondrial metabolism, whereas wild-type (WT)-PTEN GBM cells have a higher mitochondrial respiration capacity (Comelli et al., 2018).

It seems therefore that in GBM, the EGFR-PTEN axis can coordinate an enhanced glycolytic flux with attenuated mitochondrial metabolism, and this may be, in part, regulated by PGK1 (Fig. 1). Adding another layer of regulation, it has been reported that M2 tumor-associated macrophages can also phosphorylate and activate PGK1 in GBM cells *via* secretion of interleukin 6 (IL6) (Zhang et al., 2018b) (Fig. 1). Taken together with evidence that *PGK1* gene expression is upregulated in GBM compared with a normal brain (Stanke et al., 2021) and that phosphorylation levels of PGK1 correlate with GBM grade and survival (Zhang et al., 2018b), PGK1 lends itself as a potential therapeutic target in GBM especially in the context of PTEN loss. Supporting a potential therapeutic impact of PGK1 targeting, Qian et al. (2019) observed decreased

glycolytic flux in the context of PTEN loss when a mutated PGK1 (Y324F) was expressed to prevent autophosphorylation, thus mimicking a knockdown of PGK1 glycolytic activity.

EGFR-PTEN axis and the PKM2. Another glycolytic enzyme that is regulated by EGFR signaling is PKM2 (Fig. 1). Whereas other isoforms of PK exist in a constitutively active conformation, the M2 isoform is allosterically regulated and can shape-shift between an active (tetramer) or inactive (monomer or dimer) conformation, which can effectively constrain lower glycolysis. Paradoxically, increased glycolysis is often accompanied with the expression of the PKM2 isoform. In GBM, *PKM2* gene and protein expression increases with grade (Mukherjee et al., 2013), indicating a supportive role for tumor growth and aggressiveness.

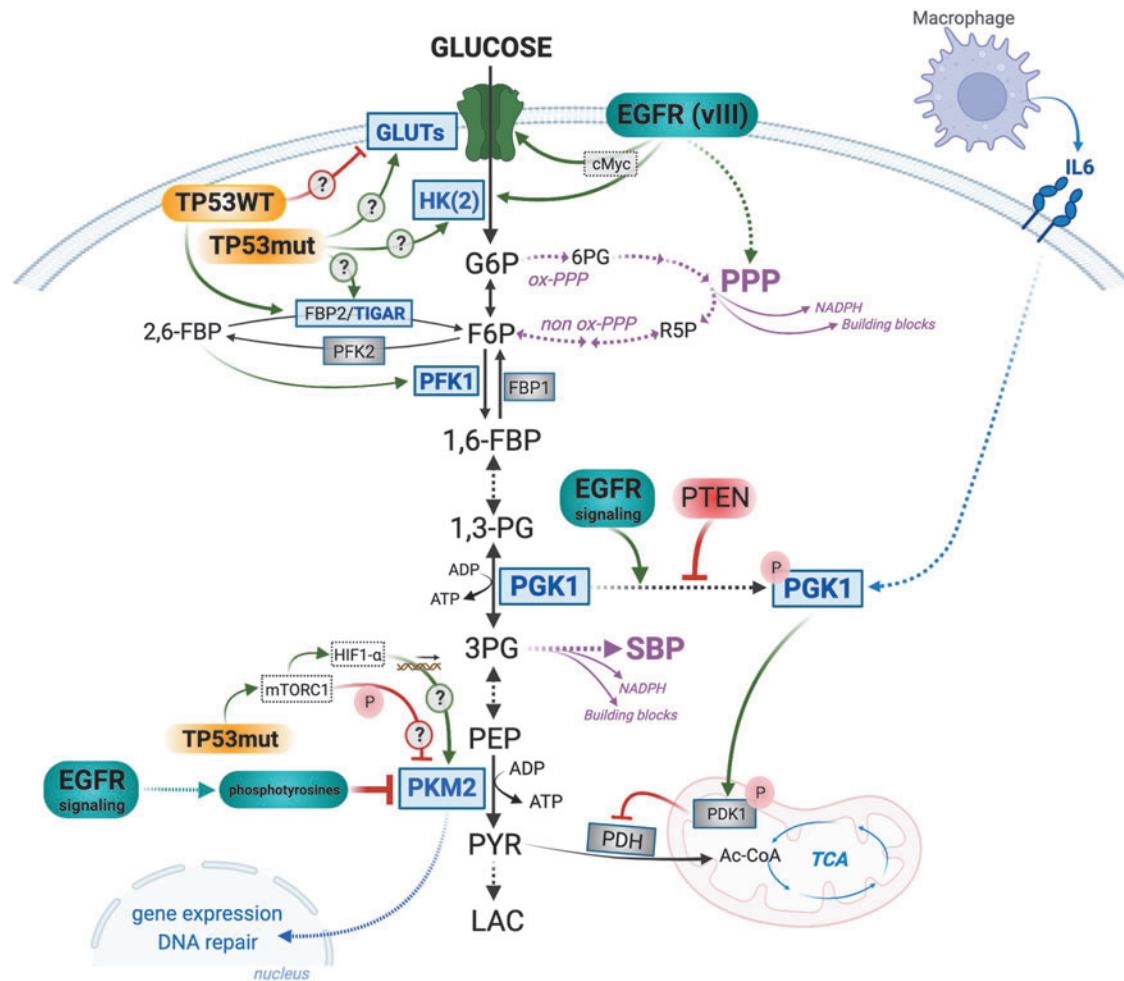
PKM2 activity is inhibited by growth factor signaling (Hitosugi et al., 2009; Li et al., 2016a; Yang et al., 2012; Yang et al., 2011) *via* binding to phosphotyrosine-marked proteins, such as EGFR target proteins (Christofk et al., 2008). In GBM, ERK1/2 kinases downstream of EGFR phosphorylate PKM2 (S37), thus inhibiting its enzymatic activity and promoting its nuclear translocation, and glycolytic gene expression (Yang et al., 2012). Comprehensive metabolic profiling has revealed increased levels of phosphoenolpyruvate (PEP) in mesenchymal gliomas, which correlates with the decreased enzymatic activity of PKM2 (Chinnaiyan et al., 2012), suggesting that hyperactivated EGFR signaling in GBM might sustain the suppression of PKM2 activity (Fig. 1).

This would be advantageous for GBM cells, as the glycolytic bottleneck created by suppressed PKM2 activity results in accumulation of upstream metabolites that become available for use in anabolic glycolytic branches, such as *de novo* serine biosynthesis pathway (SBP) or the pentose phosphate pathway (PPP), having consequences for therapy resistance (Fig. 1) (see more below). In line with this, Chinnaiyan et al. (2012) have reported higher levels of PPP metabolites, namely 6-phosphogluconate (6PG) and ribose-5-phosphate (R5P), in the metabolic signature of GBM samples, independently of their subtype, suggesting an increased need for R5P for nucleotide synthesis (Fig. 1).

PKM2, similar to PGK1, also has moonlighting functions distinct from its canonical role as a glycolytic enzyme (İlhan, 2022). Glycolytically *inactive* PKM2 translocates to the nucleus where it regulates expression of genes (Gao et al., 2012; Lee et al., 2008; Luo et al., 2011; Sizemore et al., 2018; Wang et al., 2014; Yang et al., 2012; Yang et al., 2011) such as  $\beta$ -catenin-target genes (Yang et al., 2011), hypoxia-inducible factor (*HIF*)1 (Luo et al., 2011), and *TP53* (Xia et al., 2016). Although protein kinase activity has been attributed to PKM2, this remains controversial (Hosios et al., 2015). There is evidence that such moonlighting activities of PKM2 might promote therapy resistance. For example, following RT of GBM, PKM2 is phosphorylated by ataxia telangiectasia mutated (ATM) and accumulates in the nucleus where it promotes homologous recombination repair mechanisms that can promote radiation resistance (Sizemore et al., 2018) (Fig. 1).

#### *The potential for TP53 pathway alterations in rewiring (GBM) glucose metabolism*

Mutations in the tumor suppressor gene *TP53*, and its various pathway regulators, are found in more than 80% of



**FIG. 1. Role of oncogenic mutations in promoting altered glucose metabolism.** Glucose is transported into the cell *via* GLUTs. Once inside the cell, glucose is phosphorylated by HKs into G6P in the first, irreversible step of the glycolytic pathway, a modification that traps glucose inside the cell. G6P reversibly isomerizes into F6P, which is then converted into 1,6-FBP by PFK1. Alternatively, PFK2 can convert F6P to 2,6-FBP, which is a potent allosteric activator of PFK1. Opposing the PFKs, FBPs (FBP1 and FBP2) catalyze the dephosphorylation of 1,6-FBP/2,6-FBP back to F6P. These early glycolytic steps can be affected by mutations in the EGFR and TP53 pathways. There is evidence that mutant TP53 (TP53mut) can induce the expression of TIGAR, a TP53WT-regulated bisphosphatase. The first ATP-producing enzyme in glycolysis, PGK1, converts 1,3-PG to 3PG. 3PG is the initiating metabolite for the SBP. PTEN can dephosphorylate PGK1. Under a fine regulation by the EGFR/PTEN axis, PGK1 can translocate to the mitochondria where it regulates the TCA cycle *via* PDK1 activation and PDH inhibition. M2 tumor-associated macrophages can also phosphorylate and activate PGK1 *via* secretion of IL6. The PKM2, which catalyzes the last rate-limiting step in glycolysis converting PEP to PYR and making ATP in the process, is also regulated by phosphotyrosines on proteins phosphorylated *via* EGFR signaling, and by the TP53 pathway alterations, through HIF1 $\alpha$  and mTORC1. PKM2 inhibition can lead to a bottleneck in glycolysis and rerouting of upstream metabolites into the oxidative and nonoxidative PPP. Enzymatically inactive PKM2 exhibits moonlighting functions and can regulate gene expression in the nucleus including genes responsible for DNA repair proteins. EGFR signaling represents the activation of EGFR and downstream signaling. *Question marks* refer to studies performed in tumor models other than GBM. *Green arrows* indicate activation, while *red arrows* indicate inhibition. 1,3-PG, 1,3-bisphosphoglycerate; 1,6-FBP, fructose-1,6-bisphosphate; 3PG, 3-phosphoglycerate; EGFR, epidermal growth factor receptor; F6P, fructose-6-phosphate; FBP, fructose bisphosphatase; G6P, glucose-6-phosphate; GBM, glioblastoma; GLUT, glucose transporter; HIF, hypoxia-inducible factor; HK, hexokinase; IL6, interleukin 6; mTORC1, mammalian target of rapamycin complex 1; PDH, pyruvate dehydrogenase complex; PDK1, pyruvate dehydrogenase kinase 1; PEP, phosphoenolpyruvate; PFK, phosphofructokinase; PGK1, phosphoglycerate kinase 1; PKM2, M2 isoform of pyruvate kinase; PPP, pentose phosphate pathway; PTEN, phosphatase and tensin homolog deleted on chromosome 10; PYR, pyruvate; SBP, serine biosynthesis pathway; TCA, tricarboxylic acid; TIGAR, TP53-induced glycolysis and apoptosis regulator; WT, wild-type.

GBM tumors, although they do not correlate with GBM survival (Zhang et al., 2018a). The frequency of direct mutations in the *TP53* gene varies between the different GBM molecular subtypes, with the proneural subtype having the highest (~50%), while the classical ones lack *TP53* mutations (Verhaak et al., 2010). Whereas *TP53* deletions are common in other tumor types, missense mutations with loss of transcription factor activity dominate in GBM. These missense mutations often give rise to the oncogenic “gain-of-function” *TP53* protein mutant (*TP53mut*) that can promote proliferation and resistance to apoptosis (Dittmer et al., 1993).

The six most frequent missense or “hotspot” mutations account for 25% of *TP53* mutations across all tumor types (Zhang et al., 2018a). There is a dearth of studies on the role of *TP53* pathway alterations on glucose metabolism in GBM, but studies on other tumor models provide some insight on the potential metabolic consequences of *TP53* mutations.

Using lung and breast cancer cells, Zhang et al. (2013) observed that introduction of several “hotspot” mutations in the *TP53* gene increases glucose uptake and lactate production, akin to a Warburg effect, whereas knocking down endogenous *TP53mut* reversed this phenotype. Mechanistically, *TP53* hotspot mutations, through rho-associated protein kinase (ROCK) signaling, promote plasma membrane translocation of GLUT1 enhancing glucose uptake (Zhang et al., 2013). In contrast, in osteosarcoma cells, WT *TP53* (*TP53WT*) downregulates GLUT1 expression by direct transcriptional repression, whereas mutations in the binding domain of *TP53* prevents its binding to the *SLC2A1* promoter abrogating transcriptional repression (Schwartzberg-Bar-Yoseph et al., 2004). It seems, therefore, that alterations that suppress *TP53WT* transcriptional regulation will likely lead to a higher availability of GLUTs and an enhanced capacity for taking up glucose by tumor cells, especially in the context of hotspot *TP53* mutations (Fig. 1).

Once glucose is transported into the cell, it is phosphorylated by HKs into glucose-6-phosphate (G6P), a modification that traps glucose inside the cell. HK2 is the predominant isoform of HK expressed in GBM relative to LGG and normal brain, which generally express HK1 (Wolf et al., 2011). The effect of *TP53mut* on HK levels in GBM remains unknown, but there is evidence in hepatoma cells that *TP53mut* binds to *TP53* motifs in the *HK2* promoter and increases its expression (Mathupala et al., 1997) (Fig. 1).

Following phosphorylation by HKs, G6P reversibly isomerizes into fructose-6-phosphate (F6P), which is then converted into fructose-1,6-bisphosphate (1,6-FBP) by phosphofructokinase 1 (PFK1). Alternatively, PFK2 can convert F6P to 2,6-FBP, which is a potent allosteric activator of PFK1. Opposing the PFKs, fructose bisphosphatase (FBP1 and FBP2) catalyze the dephosphorylation of 1,6-FBP/2,6-FBP back to F6P, thus tightly regulating flux through glycolysis (Fig. 1). The *TP53*-induced glycolysis and apoptosis regulator (TIGAR), a *TP53WT*-induced protein (Bensaad et al., 2006), has bisphosphatase activity and can reduce glycolytic flux by degrading 2,6-FBP, the activator of PFK1. This results in the use of upstream glycolytic metabolites by the PPP to generate intermediates for nucleotide synthesis and the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) (Bensaad et al., 2006) (see more below).

It is no surprise, therefore, that TIGAR plays an important role in promoting cancer growth, including in GBM where its overexpression is associated with enhanced growth, lower

oxidative stress, reduced overall survival (OS), and resistance to RT and TMZ (Tang and He, 2019). The effect of *TP53mut* on TIGAR is not clear in GBM although transcriptionally active *TP53mut*, such as R175P, has the potential to induce TIGAR expression (Bensaad et al., 2006) with metabolic responses that can affect therapeutic efficacy.

*TP53* can also regulate PKM2 at the transcriptional and enzymatic activity levels. Although not shown in GBM, in pancreatic cancer, *TP53mut* (R273H) can stimulate mammalian target of rapamycin complex 1 (mTORC1) activity, most likely through growth factor receptors upstream of mTORC1 (Dando et al., 2016). This leads to mTORC1-induced phosphorylation of PKM2, a modification that prevents tetramer formation and reduces its pyruvate kinase activity (Hitosugi et al., 2009) (Fig. 1). mTOR can also increase HIF1- $\alpha$  expression, which can activate the transcription of *PKM2* in different tumor models (Sun et al., 2011) (Fig. 1).

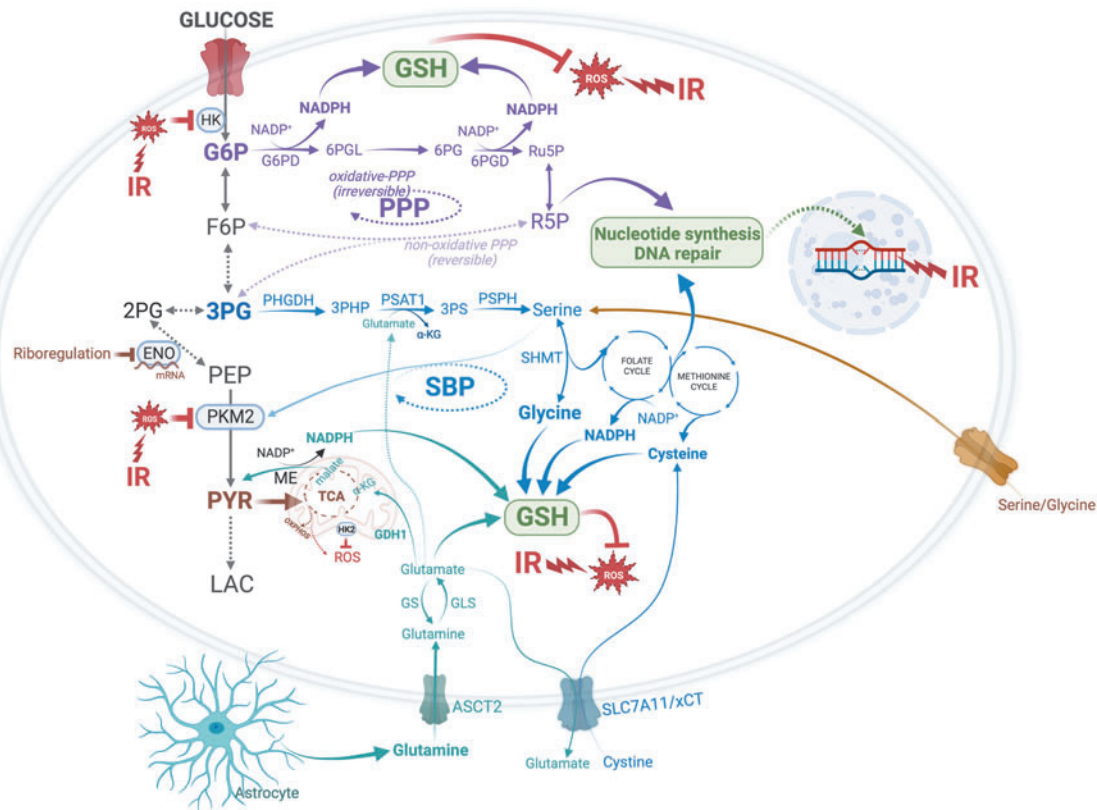
The above studies, largely performed in tumor types other than GBM, point to the potential for various *TP53* mutations to directly impact GBM glucose metabolism (question marks on Fig. 1). In line with this, in a GBM-specific study, Mai et al. (2017) show that a fraction of GBM patient-derived lines respond to EGFR inhibition by decreasing glucose consumption, a response that primes them for apoptosis in a (cytoplasmic) *TP53WT*-dependent manner. The GBM cells with hotspot *TP53* mutations that decrease their glucose consumption following EGFR inhibition seem to lack this “apoptotic priming” (Mai et al., 2017). Clearly, *TP53* mutational status exerts a complex regulation on GBM metabolism that requires context-specific investigations.

### Glycolytic Branches: A Reservoir of Building Blocks and Antioxidant Defenses That Can Promote Therapy Resistance

As the cell oxidizes glucose *via* glycolysis, the resulting intermediates are often diverted away from glycolysis to fulfill other metabolic needs. For rapidly proliferating cancer cells, a priority is generating enough energy and building blocks to support cell growth and division while keeping in check the oxidative stress that accompanies enhanced metabolic activity. Oxidative stress happens to also be one of the primary targets of RT. A collapse of the redox state would significantly disrupt biochemical reactions, a potentially lethal situation, making its stabilization a major requirement for cancer cells during RT. One central balancing mechanism is the production of reducing equivalents in the form of NADPH, a main cofactor of antioxidant systems. NADPH can neutralize oxidative stress either directly by buffering reactive oxygen species (ROS) or indirectly by recycling oxidized glutathione (GSSG) into its reduced form (GSH).

As demonstrated by recent studies, the ability of cancer cells to reconfigure metabolic fluxes to optimize the production of reduced redox cofactors seems to be critical in resisting the cytotoxic effects of RT (Lewis and Kemp, 2021; Lewis et al., 2021).

Three glycolytic intermediates feed into auxiliary pathways that ultimately provide the energy, building blocks and reducing equivalents needed for uninhibited proliferation and maintenance of redox homeostasis: G6P feeds the oxidative PPP; 3-phosphoglycerate (3PG) feeds into the *de novo* SBP; and pyruvate feeds into the TCA cycle (Fig. 2). How common



**FIG. 2. Role of glycolysis and branching pathways in therapy resistance.** As the cell oxidizes glucose *via* glycolysis, the resulting intermediates are often diverted away from glycolysis to fulfill other metabolic needs. For rapidly proliferating cancer cells, a priority is generating enough energy and building blocks to support cell growth and division while keeping in check the oxidative stress that accompanies enhanced metabolic activity. Oxidative stress is also one of the primary targets of RT. G6P feeds the oxidative PPP, which generates precursors (R5P) for nucleotide synthesis and DNA repair. PPP also generates NADPH, *via* G6PD and 6PGD. HK2, responsible for glucose phosphorylation, can also protect from oxidative stress through binding to the mitochondria membrane to prevent mitochondrial ROS production. 3PG feeds into the *de novo* SBP, which also generates building blocks for glutathione and nucleotide synthesis, and more NADPH. 3PG is converted into serine *via* three consecutive enzymatic reactions, catalyzed by PHGDH, PSAT1, and PSPH. In a reversible reaction, serine is converted to glycine *via* SHMT1/2. During the conversion of serine to glycine, SHMT concomitantly charges 1CM with one carbon units. 1CM couples the folate and methionine cycles, which generate amino acids, nucleotides, lipids, and reducing equivalents in the form of NADPH. Cancer cells can also take up serine and glycine from exogenous sources. PYR, produced by PK(M2), feeds into the TCA cycle or the FA synthesis pathway providing additional energy and building blocks for nucleic acids, proteins, and membranes. Glutamine, made by astrocytes, can be used by glioma cells and enters the cell *via* the amino acid antiporter ASCT2 and is converted to glutamate by GLS, which participates in GSH synthesis and antioxidant defenses. Glutamate can also be converted to  $\alpha$ -KG by GDH1 thus replenishing the TCA cycle. Malate produced in the TCA cycle can be converted to PYR *via* the malic enzyme and NADPH is produced in the process. Glutamine can be converted back to glutamine *via* GS or exported outside the cell as an exchange for cystine (the oxidized dimer form of the amino acid cysteine) *via* the SLC7A11/xCT. 1CM, one-carbon metabolism; 6PGD, 6-phosphogluconate dehydrogenase;  $\alpha$ -KG,  $\alpha$ -ketoglutarate; ASCT2, Alanine, Serine, Cysteine Transporter 2; FA, fatty acid; G6PD, glucose-6-phosphate dehydrogenase; GDH1, glutamate dehydrogenase-1; GLS, glutaminase; GS, glutamine synthetase; GSH, reduced glutathione; NADPH, reduced nicotinamide dinucleotide phosphate; PHGDH, phosphoglycerate dehydrogenase; PSAT1, phosphoserine aminotransferase 1; PSPH, phosphoserine phosphatase; R5P, ribose-5-phosphate; ROS, reactive oxygen species; RT, radiation therapy; SHMT, serine hydroxymethyl-transferase; SLC7A11/xCT, cystine/glutamate antiporter.

oncogenes and tumor suppressor genes regulate these pathways in cancer cells in general has recently been reviewed (De Santis et al., 2018). In this section, we focus on the role of the PPP and the SBP in promoting tumor growth and therapy resistance, highlighting GBM-relevant studies whenever possible.

#### The PPP

The functions of PPP are twofold, to provide phosphopentoses for synthesis of nucleotides and to generate reducing

equivalents in the form of NADPH *via* the oxidative arm of the PPP (Fig. 2). Nucleotides are necessary for DNA replication and repair, whereas NADPH contributes to reductive biosynthesis of lipids and is essential for maintaining redox homeostasis (Patra and Hay, 2014). A shift in primary carbon metabolism to enhance the PPP is the fastest way to produce NADPH, and therefore, activation of the PPP is a known metabolic response in protecting eukaryotic cells from oxidative stress (Filosa et al., 2003; Kuehne et al., 2015; Ralser et al., 2007). There is evidence, in normal cells and yeast

systems, that shifting of glycolytic flux toward the PPP can occur within seconds of exposure to oxidative stress in a transcription-independent manner, followed by a gene expression-dependent antioxidant response (Kuehne et al., 2015; Ralser et al., 2009).

The initial acute response is thought to be mediated by the inhibition of the enzymatic activity of downstream redox-sensitive glycolytic enzymes, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Ralser et al., 2009) and PKM2 (Anastasiou et al., 2011), which create a block in lower glycolysis making upstream metabolites available for the PPP. Others have reported, however, that this can also happen independently of GAPDH and PKM2 inhibition by oxidative stress (Kuehne et al., 2015). In the latter study, Kuehne et al. (2015) report that NADPH is a direct inhibitor of glucose-6-phosphate dehydrogenase (G6PD), the first rate-limiting enzyme in the PPP, and that acute depletion of NADPH by oxidative stress, in this case H<sub>2</sub>O<sub>2</sub> or ultraviolet, removes the inhibitory effect on G6PD thus enhancing flux through the PPP.

HKs are redox-sensitive enzymes inhibited by oxidative stress (Heneberg, 2019) (Fig. 2) and there is some evidence that they regulate NADPH levels in ovarian cancer cells (Šimčíková et al., 2021), however, it remains unclear whether this is through the PPP. Although not performed in GBM, studies show that binding of HK2, the main isoform in GBM, to the mitochondria membrane protects cells from oxidative stress by reducing ROS generated in the mitochondria (da-Silva et al., 2004; Sun et al., 2008) (Fig. 2).

GAPDH is present in all normal cells, whereas PKM2 tends to be preferentially expressed in cancers and in GBM its expression increases with grade (Mukherjee et al., 2013). In a lung cancer model, oxidative stress induces oxidation of Cys<sup>358</sup> residue on PKM2 and inhibition of its enzymatic activity (Anastasiou et al., 2011). Others have identified additional oxidation events that inhibit PKM2 activity (Irokawa et al., 2021). We have shown a similar inhibitory effect induced by oxidative stress generated during radiation of breast cancer cells (Zhang et al., 2019). In the lung cancer model (Anastasiou et al., 2011), oxidative stress-induced inhibition of PKM2 results in increased flux through the PPP and enhanced NADPH production that supports a robust antioxidant response, promoting survival under oxidative stress conditions (Anastasiou et al., 2011) (Fig. 2).

In parallel, the PPP generates precursors for nucleotides, which are essential for repairing DNA damage (Fig. 2). It seems, therefore, that rewiring glycolytic flux to enhance the PPP would be of particular importance in the context of RT in GBM where purine metabolism has been associated with radiation resistance (Zhou et al., 2020).

Two enzymes in the PPP directly contribute to the NADPH pool, the first enzyme, G6PD, and the third enzyme, 6-phosphogluconate dehydrogenase (6PGD) (Fig. 2). It is reported that 6PGD is upregulated in GBM compared with normal tissue, whereas G6PD is lower (Stanke et al., 2021). Pointing to a tumor promoting role for these enzymes, analysis of The Cancer Genome Atlas (TCGA) data sets (Goldman et al., 2020) on gliomas reveals that gene expression levels of both enzymes are highly correlated with OS of LGG (Fig. 3A, B), whereas in GBM the gene expression levels of only G6PD are correlative, trending toward significance for OS (sample numbers with gene expression data are much

lower in the GBM cohort) (Fig. 3C), but significantly correlating with progression-free survival (Fig. 3D).

### The SBP

The nonessential amino acid serine contributes to numerous biosynthetic pathways and its role in metabolic plasticity in cancer is increasingly being appreciated (Geeraerts et al., 2021). Cells can obtain serine *via* multiple ways, including *de novo* synthesis from glucose, conversion from glycine, catabolism from proteins and serine-containing phospholipids (PLs), and by transporter-mediated uptake from the extracellular environment (de Koning et al., 2003). However, some cancers, such as aggressive breast cancer, become addicted to *de novo* production of serine (Possemato et al., 2011). In the normal brain, serine is critical for neurotransmission and is newly synthesized by glial cells *via* the *de novo* SBP (Maugard et al., 2021). 3PG is converted into serine *via* three consecutive enzymatic reactions, catalyzed by phosphoglycerate dehydrogenase (PHGDH), the first rate-limiting enzyme in the SBP, phosphoserine aminotransferase 1 (PSAT1), and phosphoserine phosphatase (PSPH).

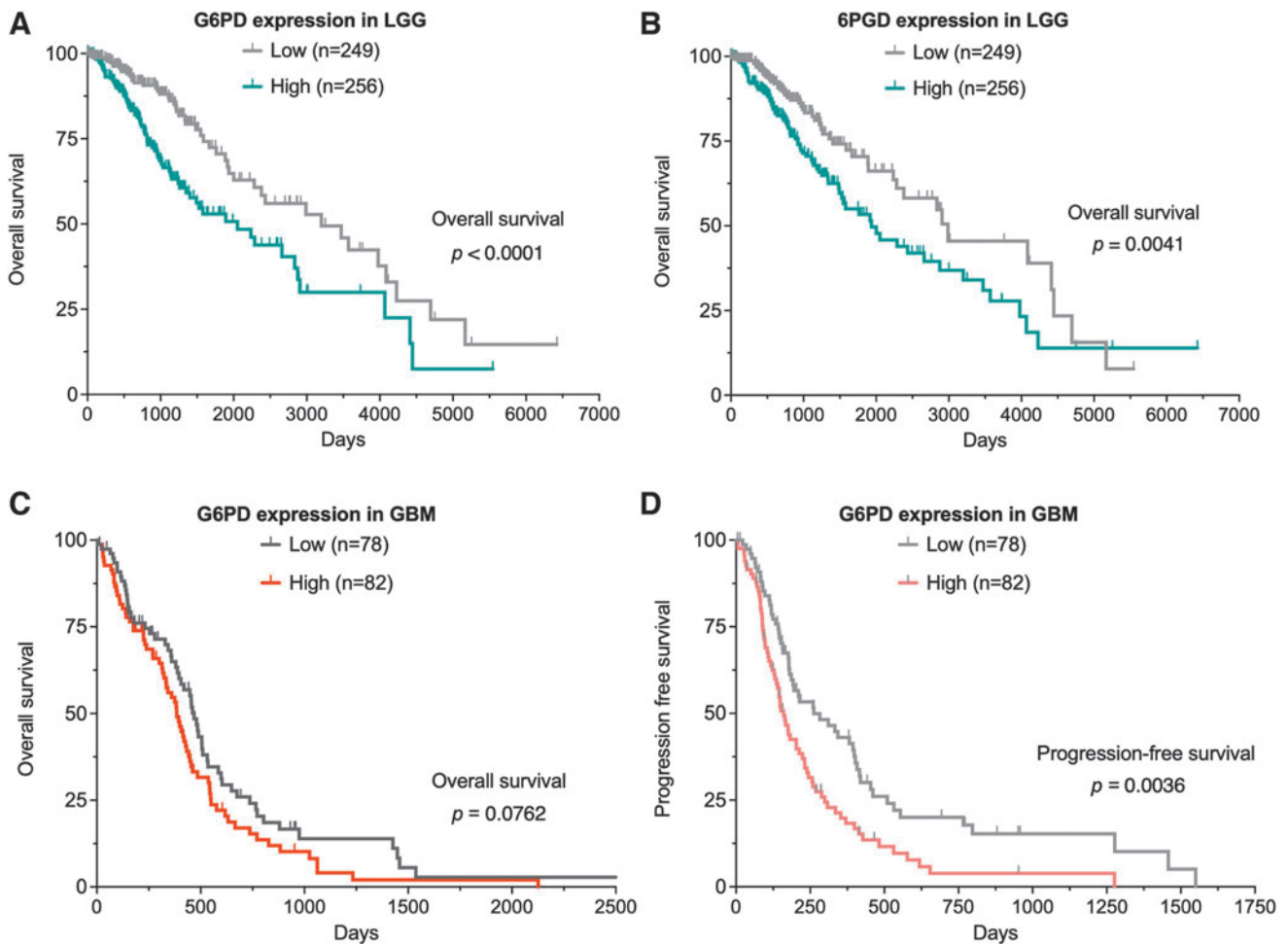
In a final reversible step, serine is converted to glycine *via* serine hydroxymethyl transferases, SHMT1/2 (cytoplasmic/mitochondrial), concomitantly charging one-carbon metabolism (ICM) with one carbon units (Fig. 2). ICM couples the folate and methionine cycles, which generate amino acids, nucleotides, lipids, and NADPH (Fig. 2). Flux into the SBP can be regulated by serine itself, which is an allosteric activator of PKM2, thus restricting 3PG routing into the SBP when serine levels are high (Fig. 2). Enolase (ENO) generates 3PG from 2PG. ENO1 seems to promote cell growth and invasion in GBM (Song et al., 2014) and a subset of gliomas harbor homozygous deletion of ENO1 that makes them vulnerable to the inhibition of its paralogue ENO2 (Lin et al., 2020).

Mechanistically, a recent study shows that ENO1 binds cellular messenger RNAs (mRNAs) resulting in inhibition of its enzymatic activity and rewiring of glycolysis. One of the consequences is increased synthesis of serine *via* the SBP (Huppertz et al., 2022) (Fig. 2).

While it is generally accepted that the largest contributor to cytosolic NADPH in proliferating cells is the PPP, a comprehensive study quantifying NADPH fluxes demonstrated that the serine-driven ICM generates NADPH at levels comparable with those in the PPP (Fan et al., 2014). SBP also generates the precursors for glutathione, which comprised glycine, cysteine, and glutamate (derived from glutamine). GSH synthesis, therefore, is sustained through direct uptake and/or *de novo* synthesis of serine and glycine and the generation of cysteine through the methionine cycle of ICM that directly consumes serine (Fig. 2). Analysis [*via* cBioPortal (Cerami et al., 2012; Gao et al., 2013)] of TCGA data sets on LGG and GBM revealed the presence of copy number gains and amplifications in all the SBP enzymes in both tumor types, but an increased frequency in GBM (Fig. 4).

Copy number gains and amplifications occur with the greatest frequency in the *PSPH* gene with a dramatic increase in GBM. ~80% of GBM have copy number gains and ~12% have copy number amplifications (Fig. 4). Copy number variations (CNVs, include deletions) in *PHGDH* correlate with OS in only LGG and not GBM (Fig. 5A, B),





**FIG. 3. Gene expression levels of NADPH-producing enzymes in the PPP correlate with survival in GBM.** TCGA data set analysis on GBMs reveals that gene expression levels of G6PD and 6PGD are highly correlated with the overall survival of LGG (A, B), whereas in GBM, the gene expression levels of only G6PD trend toward significance ( $p = 0.0762$ ) for correlating with overall survival (C), but it significantly correlates with progression-free survival (D). Data sets were analyzed in GraphPad Prism software and  $p$ -values determined *via* log-rank (Mantel–Cox) test. LGG, low-grade gliomas; TCGA, The Cancer Genome Atlas.

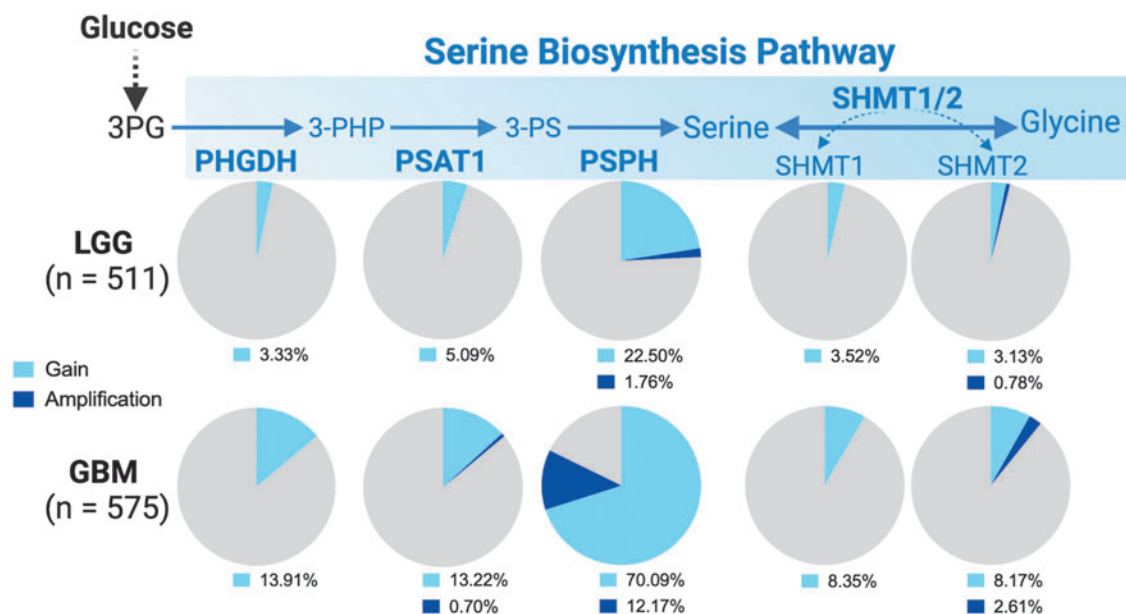
whereas CNVs in the *PSPH* gene significantly correlate with OS in both LGG and GBM (Fig. 5C, D). Interestingly, although CNVs are highly correlated with *PSPH* gene expression levels in both LGG and GBM (Fig. 5E, F), only copy numbers significantly correlate with OS (Fig. 5C, D), whereas gene expression levels do not (not shown).

A recent study offers some insight into the curiously low copy number gains and lack of amplifications in the rate-limiting enzyme PHGDH, relative to the other SBP enzymes (Fig. 4). It seems that higher PHGDH levels, at either the gene expression or protein level, correlate with a favorable prognosis in GBM. This suggests a functional role of PHGDH in limiting tumor aggressiveness and thus selection for PHGDH-low cells during tumor evolution (Oh et al., 2020).

Although low in frequency, copy number amplifications are only found in the SHMT2 isoform (mitochondrial) and not the SHMT1 (cytoplasmic) (Fig. 4), pointing to a potentially greater importance of SHMT2 over SHMT1 in GBM. In line with this, recently, Engel et al. (2020) showed that under hypoxic conditions, GBM cells upregulate SHMT2 but not SHMT1, indicating that SHMT2 plays a crucial role in the

survival of hypoxic GBM cells, such as in areas of pseudopalisading, which is common in GBM (Evans et al., 2004). Adding mechanistic insight, another study connected high levels of SHMT2 with lower PKM2 activity and hypothesized that lower PKM2 activity limits flux into the TCA cycle resulting in decreased oxygen consumption and enhanced GBM cell survival under hypoxia (Kim et al., 2015).

Mitochondrial folate metabolism has been linked to cancer development (Yang and Vousden, 2016). Under starvation conditions, as in the core of GBM tumors, for example, PSAT1, the mitochondrial methylenetetrahydrofolate dehydrogenase (MTHFD2) and serine-dependent ICM are upregulated to counteract the effects of nutrient deprivation and allow GBM cells to survive harsh microenvironmental conditions. In tumors with TP53 mutations or loss, including GBM, MTHFD2 is often upregulated and required for cancer cell proliferation and DNA repair (Li et al., 2021), pointing to a potential mechanism for radiation resistance in TP53mut GBMs. Surprisingly little is known about the effect of localized RT-induced oxidative stress on serine metabolism in GBM. This warrants further investigation, as funneling glucose carbons



**FIG. 4. Pathway alterations in the SBP in GBM.** TCGA data set analysis on LGG and GBM reveals the presence of copy number gains and amplifications in all the SBP enzymes in both tumor types, but an increased frequency in GBM. 3PG, derived from glucose, is converted into serine *via* three consecutive enzymatic reactions, catalyzed by PHGDH, PSAT1, and PSPH. In a final reversible step, serine is converted to glycine *via* SHMTs, SHMT1 (cytoplasmic) or SHMT2 (mitochondrial). 3PHP, 3-phosphohydroxypyruvate, 3PS, 3-phosphoserine.

into the *de novo* SBP might be an alternative, or even complementary, pathway *via* which irradiated GBM cells support a robust antioxidant response (Engel et al., 2020), DNA repair (Sánchez-Castillo et al., 2021), and resistance to RT.

#### Glutamine Metabolism Facilitates Therapy Resistance by Replenishing Antioxidant and Nucleotide Pools

Another important source of energy and antioxidants for cancer cells is the catabolism of glutamine. Glutamine, a nonessential amino acid, is the most abundant free amino acid in human blood (Brosnan, 2003). Despite this, astrocytes in the normal brain have high expression of glutamine synthetase (GS) (Tardito et al., 2015) and can synthesize glutamine *de novo* from glutamate (Bagga et al., 2014), which is derived from extracellular sources or formed from  $\alpha$ -ketoglutarate ( $\alpha$ -KG) in the TCA cycle. GBM cells mainly obtain glutamine from *in situ* sources, taking advantage of astrocytic synthesis (Tardito et al., 2015), with only 20% maximum originating from the circulation in the normal mouse brain (Bagga et al., 2014). Glutamine enters the cell *via* the amino acid antiporter ASCT2 (Alanine, Serine, Cysteine Transporter 2) (Scalise et al., 2018) and is converted to glutamate by glutaminase (GLS) (Fig. 2).

Glutamate can then participate in numerous cellular processes, including glutathione synthesis, SBP, and anaplerosis, defined as the replenishment of metabolites in the TCA cycle (Owen et al., 2002) (Fig. 2). Glutamine metabolism is therefore intimately interconnected with the cell's antioxidant systems. GBM glutamine metabolism and targeting opportunities have been recently reviewed (Obara-Michlewska and Szeliga, 2020).

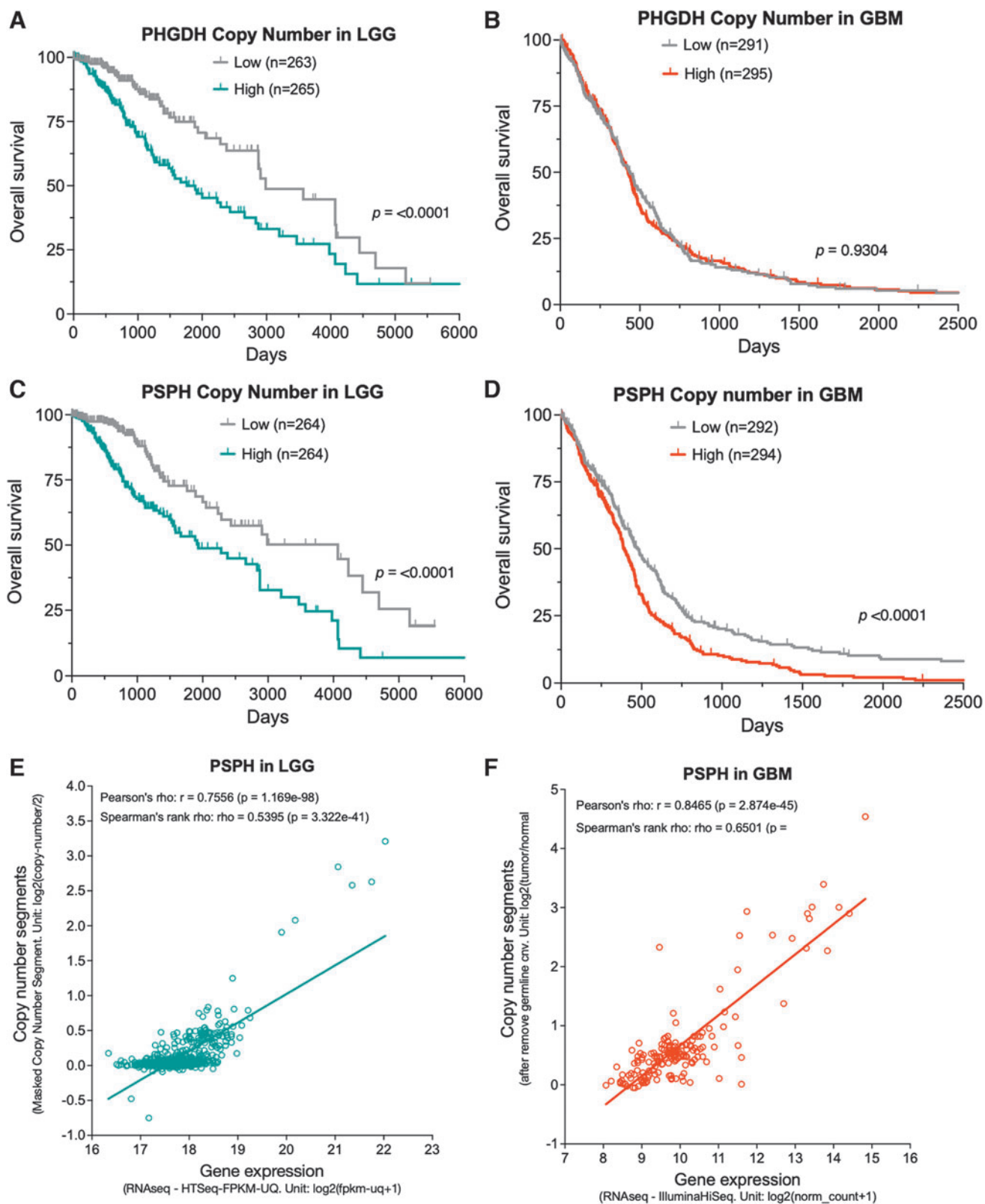
Glutamine's contribution not only to the carbon pool but also as a nitrogen source makes it indispensable for many

tumor types, including triple-negative breast cancer (Quek et al., 2022), lung cancer (Hassanein et al., 2015), pancreatic cancer (Son et al., 2013), and as discussed below, in GBM.

The dependence on glutamine seems to be heterogeneous across GBM subtypes, as different cell lines have differing sensitivities to glutamine starvation (Tardito et al., 2015). Mesenchymal GBMs, for example, increase glutamine uptake and utilization (Oizel et al., 2020; Oizel et al., 2017), whereas GS expression seems to be restricted to non-mesenchymal GBM (Oizel et al., 2020). GBM tumor tissue accumulates a large pool of intracellular glutamine in orthotopic GBM models due to higher synthesis and import (Marin-Valencia et al., 2012). However, it remains unclear if glutamine is used by GBM tumors as a mean to support anaplerosis. Some studies have shown an enrichment of glutamine-derived carbons in the TCA cycle of GBM cells compared with normal brain (Oizel et al., 2020) associated with increased NADPH production most likely *via* the malic enzyme (DeBerardinis et al., 2007) (Fig. 2).

DeBerardinis et al. (2007) have demonstrated that GBM cells use glutamine for the synthesis of fatty acids and aspartate, a precursor for nucleotide synthesis and asparagine/arginine synthesis. However, others suggest that GBM tumors mainly use glucose to supply the TCA cycle (Marin-Valencia et al., 2012) and that glutamine-induced anaplerosis is not essential for GBM cell proliferation (Tardito et al., 2015).

A recent study showed that the EGFR pathway promotes glutamate dehydrogenase-1 (GDH1) transcription in GBM (Yang et al., 2020), thereby stimulating glutamine metabolism. GDH1 catalyzes the formation of  $\alpha$ -KG from glutamate (Fig. 2), suggesting that GDH1 upregulation promotes anaplerosis. Introduction of EGFR-vIII into GBM cells increases the expression of other key enzymes in glutaminolysis, such



**FIG. 5. CNVs correlate with the overall survival in LGG and GBM.** TCGA data set analysis of CNVs (include deletions) on LGG and GBM reveals that CNVs of PHGDH and PSPH correlate with overall survival, with PHGDH CNVs correlating with survival in only LGG and not GBM (**A, B**), whereas CNVs in the PSPH gene significantly correlate with overall survival in both LGG and GBM (**C, D**). CNVs are highly correlated with PSPH gene expression levels in both LGG and GBM (**E, F**). CNV, copy number variation.

as GLS and GS (Tanaka et al., 2015). GBMs often resist EGFR inhibitors, which is mediated, in part, by the mTOR pathway (Mellinghoff et al., 2005). However, mTOR inhibition fails to sensitize EGFR-vIII GBM tumors to EGFR inhibition, but rather increases glutaminolysis (Tanaka et al., 2015), pointing to glutamine metabolism as a player in therapy resistance.

Glutamate is exported *via* the cystine/glutamate antiporter (SLC7A11/xCT), in exchange for cystine (Jyotsana et al., 2022) (Fig. 2). SLC7A11/xCT is overexpressed in GBM cells relative to normal human astrocytes (Polewski et al., 2016) and glutamate export supports tumor progression in GBM (Takano et al., 2001), most likely because of the neurotoxicity of high levels of glutamate. Under glutamine starvation, GBM cells still export glutamate, but prioritize glutamine synthesis from glutamate (Tardito et al., 2015), thereby promoting cataplerosis [reactions involved in the removal of TCA cycle intermediates (Owen et al., 2002)], to fuel *de novo* purine synthesis (Tardito et al., 2015).

#### *Effects of anticancer therapies on glutamine metabolism*

Very few studies have addressed the impact of anticancer therapies on glutamine metabolism in GBM. At the transport level, xCT is associated with chemo- and radioresistance in GBM. Knocking down xCT leads to increased oxidative stress, depletion of GSH, and enhanced sensitivity to TMZ (Polewski et al., 2016). Other studies have connected glutamine metabolism to radiation resistance although without directly addressing the impact of RT on how tumors use glutamine. xCT targeting sensitizes breast cancer cells to RT due to depletion of GSH pools (Cobler et al., 2018). There is also evidence that radioresistant cancer cells, including GBM, enhance glutamine anabolism *via* upregulation of GS expression and suppress glycolysis, TCA cycle, and mitochondrial respiration (Fu et al., 2019).

The interpretation is that the boosted glutamine levels provide the necessary nitrogen for the increased need of pyrimidine and purine synthesis that ultimately facilitates DNA repair (Fu et al., 2019). Indeed, nucleotide metabolism, which largely depends on glutamine metabolism, is recognized as an actionable pathway for sensitizing cancer cells, including GBM, to RT (Fu et al., 2019; Zhou et al., 2020).

#### **It Is Not All About Glucose and Glutamine in GBM: Lipid Metabolism Has Emerged as a Key Player**

Although the normal brain relies mainly on glucose as its primary energy source, it is now widely accepted that the brain is capable of oxidizing fatty acids that cross the blood-brain barrier (BBB) (Ebert et al., 2003; Panov et al., 2014). Fatty acids are key players in energy storage and production and a major component of cellular lipids. Lipids exist in three main classes of esters—triacylglycerol (TAG), PLs, and cholesteryl esters—and can be obtained extracellularly from the diet or synthesized from glucose and glutamine carbons. Catabolism of glucose and glutamine *via* the TCA cycle provides citrate, which can be converted to acetyl-CoA, a precursor for lipogenesis (synthesis of PL and TAG), as well as cholesterol synthesis *via* the mevalonate pathway (Beloribi-Djefafia et al., 2016) (Fig. 6). Acetyl-CoA can also be generated from the breakdown of TAGs in the mitochondria, a process known as  $\beta$ -oxidation.

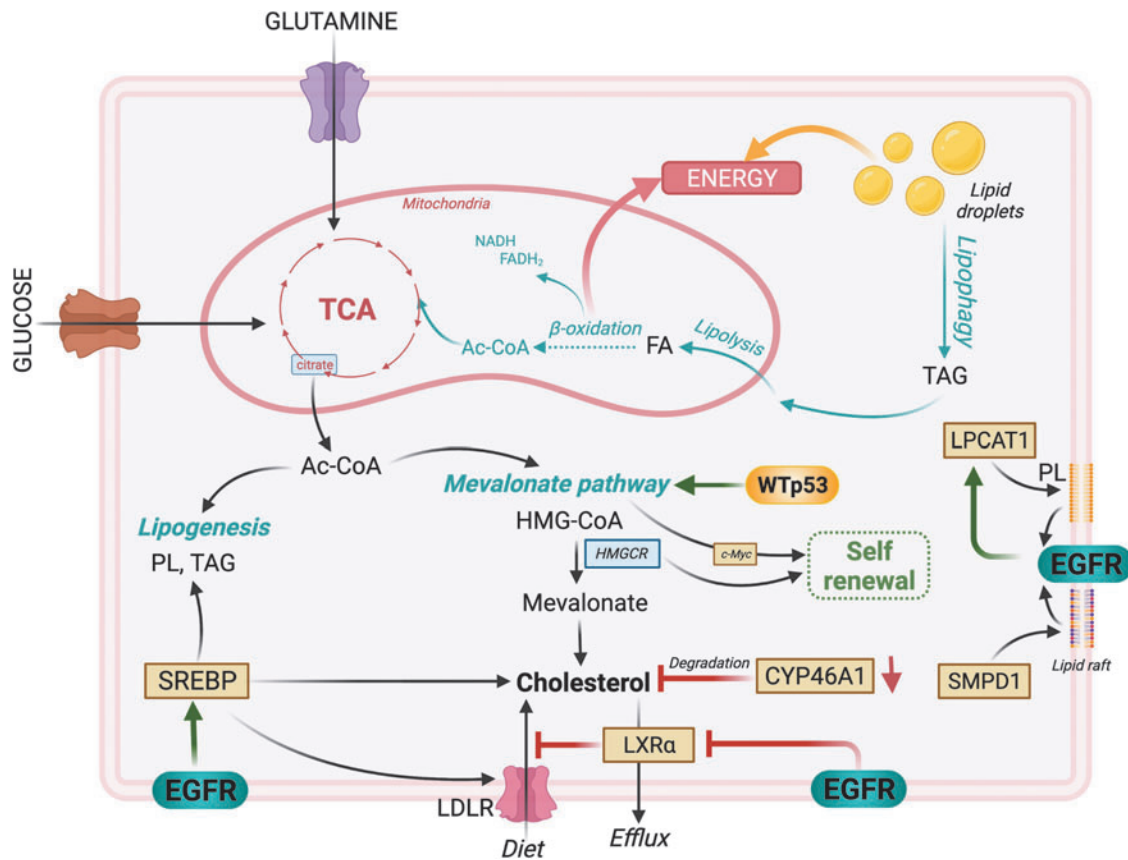
Within the cell, TAGs can also be stored inside lipid droplets, highly dynamic organelles that regulate the storage and hydrolysis of lipids. Lipid droplets are generally composed of a neutral lipid core encapsulated in a monolayer of PLs. They are fairly ubiquitous in cells and often reflect their metabolic state (Olzmann and Carvalho, 2019).

Lipid metabolism, the synthesis and degradation of lipids, has emerged as a critical contributor to tumor progression and therapy response, including in GBM (Guo et al., 2013; Shakya et al., 2021; Sperry et al., 2020; Taib et al., 2019). Rapidly proliferating cancer cells often have increased levels of lipids stored in droplets. GBM tumors display an overall increase in lipid levels compared with normal tissue (Guo et al., 2013) and further enhance lipid metabolism in a nutrient-poor environment (Shakya et al., 2021; Sperry et al., 2020). A study integrating metabolomics, lipidomics, and proteogenomics in GBM tumors revealed differences in lipid composition between GBM subtypes. The mesenchymal subtype stood out with a lipid metabolism profile distinct from other subtypes, characterized by an increased abundance of TAGs accompanied by enhanced  $\beta$ -oxidation (Kant et al., 2020; Wang et al., 2021).

Others show that human GBM tissues express fatty acid oxidation enzymes, suggesting that they can perform  $\beta$ -oxidation (Lin et al., 2017). The expression of these enzymes seems essential for aerobic respiration (Lin et al., 2017), perhaps pointing to a more important contribution of fatty acids to the TCA cycle than glucose (Sperry et al., 2020). High levels of unsaturated fatty acids in GBM, such as oleic acid, induce the accumulation of lipid droplets, which promote cell proliferation upon their hydrolysis (Taib et al., 2019) by acting as an energy reservoir (Wu et al., 2020) (Fig. 6). Although lipid droplets can be broken down *via* enzymatic hydrolysis mediated by lipases (lipolysis), lipophagy (lipid-selective autophagy) seems to be the main contributor to lipid droplet hydrolysis under glucose deprivation conditions in GBM (Wu et al., 2020) (Fig. 6).

Cholesterol, originating from acetyl-CoA *via* the mevalonate pathway, is essential for normal brain function with the brain containing almost a quarter of the body's cholesterol (Martin et al., 2014). Since the BBB prevents import of cholesterol, the brain cholesterol is synthesized locally in astrocytes and oligodendrocytes (Martin et al., 2014). Cholesterol plays an important role in tumor growth (Pirmoradi et al., 2019; Villa et al., 2016). Surprisingly, however, GBM cells lack the ability to synthesize cholesterol and must import it (Pirmoradi et al., 2019). One potential reason is that the mevalonate pathway is driven by TP53WT, and one study shows that GBM cells with a TP53mut (R273H), which lacks DNA-binding activity, downregulate several mevalonate pathway-related genes (Laezza et al., 2015) (Fig. 6).

The high prevalence of TP53 pathway alterations in GBM tumors (~80%) potentially explains their inability to synthesize cholesterol, although this might be tissue- and cell type-specific. For example, the same mutation in the *TP53* gene, in breast cancer cells, leads to the activation of the mevalonate pathway, whereas its knockdown results in decreased expression of several mevalonate pathway-related genes (Freed-Pastor et al., 2012). GBM cells may obtain cholesterol through its import from tumor-associated astrocytes (TAAs) (Perelroizen et al., 2022). TAAs accumulate at the tumor margins, produce cholesterol, and export it through



**FIG. 6. Role of lipid metabolism in therapy resistance in GBM.** Lipids exist in three main classes of esters—TAG (also referred to as triglycerides, three FAs individually esterified to each carbon of a glycerol molecule), PLs (two FAs joined to a phosphate group and an alcohol residue), and cholesteryl esters (an FA esterified to the hydroxyl group of cholesterol)—and can be obtained extracellularly from the diet or synthesized from glucose and glutamine carbons. Catabolism of glucose and glutamine *via* the TCA cycle provides citrate, which can be converted to Ac-CoA, a precursor for lipogenesis (synthesis of PL and TAG), as well as cholesterol synthesis *via* the mevalonate pathway, which can be activated by WTp53. Ac-CoA can also be generated from the breakdown of TAGs in the mitochondria, a process known as  $\beta$ -oxidation. This catabolic process generates NADH and FADH<sub>2</sub>, further utilized for ATP production. Within the cell, TAGs may also be stored inside lipid droplets, highly dynamic organelles that regulate the storage and hydrolysis of lipids. Lipid droplets are generally composed of a neutral lipid core encapsulated in a monolayer of PLs and can be broken down by lipophagy. They are ubiquitous in cells and often reflect their metabolic state. The mevalonate pathway is important for GSC self-renewal, in particular *via* HMGCR, the rate-limiting enzyme converting HMG-CoA to mevalonate. LXRs are nuclear receptors that regulate intracellular cholesterol levels by controlling the expression of genes related to cholesterol export. Upregulated EGFR signaling downregulates the expression of LXR $\alpha$  mRNA and leads to cholesterol accumulation. EGFR also induces the cleavage and nuclear translocation of SREBP, an important transcriptional regulator of FA and cholesterol synthesis. SREBP also increases cholesterol uptake by inducing the expression of LDLR. CYP46A1 converts cholesterol into 24(S)-hydroxycholesterol, thereby eliminating cholesterol. Its downregulation in GBM further increases intracellular levels of cholesterol, thus supporting growth. Upregulated EGFR signaling renders GBM cells dependent on plasma membrane remodeling, in an LPCAT1 and SMPD1-dependent manner. LPCAT1 controls membrane PL saturation, whereas SMPD1 converts sphingomyelin to ceramide and is crucial for lipid raft composition and clustering of membrane signaling molecules, such as EGFR. Ac-CoA, acetyl-CoA; CYP46A1, cholesterol 24-hydroxylase; FADH<sub>2</sub>, flavin adenine dinucleotide; GSC, GBM stem cell; HMGCR, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; LDLR, low-density lipoprotein receptor; LPCAT1, lysophosphatidylcholine acyltransferase 1; LXR $\alpha$ , liver X receptor; mRNA, messenger RNA; NADH, nicotinamide adenine dinucleotide; PL, phospholipid; SMPD1, sphingomyelin phosphodiesterase 1; SREBP, sterol regulatory element-binding protein; TAG, triacylglycerol.

the sterol transporter ATP-binding cassette transporter A1 (ABCA1). Astrocyte-derived cholesterol then promotes GBM cell survival and growth, while targeting of ABCA1 leads to tumor regression (Perelroizen et al., 2022).

There is some evidence that the mevalonate pathway maintains GBM stem cells (GSCs), which overexpress mevalonate pathway-related genes compared with differentiated GBM cells (Wang et al., 2017), suggesting a role in GBM maintenance

(Fig. 6), although the role of TP53 mutations is unclear in this context. The rate-limiting enzyme in mevalonate synthesis, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR), which converts HMG-CoA to mevalonate, is highly expressed in GSCs, in a Myc-dependent manner, and its inhibition hampers GSC self-renewal capacity and tumorigenicity *in vivo* (Wang et al., 2017), suggesting a targetable vulnerability to specifically eradicate therapy-resistant GSCs (Fig. 6).

Amplified EGFR signaling also leads to a remodeling of cholesterol metabolism in GBM (Fang et al., 2021; Gabitova et al., 2015; Guo et al., 2011; Guo et al., 2009; Villa et al., 2016). GBM cells profit from cholesterol biosynthesis in the brain to sustain their rapid growth, by maintaining high intracellular levels of cholesterol. This represents a growth advantage for GBM as cholesterol synthesis uses many nicotinamide adenine dinucleotide (NADH) reducing equivalents, which deprive other cellular processes (Lunt and Vander Heiden, 2011). GBM cells maintain their high cholesterol levels by preventing activation of liver X receptor (LXR). LXRs are nuclear receptors that regulate intracellular cholesterol levels by controlling the expression of genes related to cholesterol export. Constitutively activated EGFR signaling downregulates the expression of LXR $\alpha$  mRNA (Fang et al., 2021) (Fig. 6).

Forced activation of LXR *via* agonists negatively affects GBM growth (Guo et al., 2011; Villa et al., 2016), likely by forcing cholesterol export from the cell. In addition, EGFR induces the cleavage and nuclear translocation of sterol regulatory element-binding protein (SREBP) (Guo et al., 2009), another important transcriptional regulator of fatty acid synthesis, cholesterol synthesis, and uptake by inducing the expression of low-density lipoprotein receptor (LDLR), again leading to an increased intracellular availability of fatty acids in GBM cells (Fig. 6). Further enhancing intracellular cholesterol levels in GBM is the downregulation of cholesterol 24-hydroxylase (*CYP46A1*) gene expression. *CYP46A1* converts cholesterol into 24(S)-hydroxycholesterol, thereby eliminating cholesterol (Moutinho et al., 2016). Its overexpression or forced activation reduces cholesterol levels and decreases GBM tumor growth *in vivo* (Han et al., 2020) (Fig. 6).

Recent evidence suggests that the composition of cholesterol and PLs may be radically different in the plasma membrane of GBM cells and that this profoundly impacts EGFR signaling (Bi et al., 2021; Bi et al., 2019). Lysophosphatidylcholine acyltransferase 1 (LPCAT1) (Bi et al., 2019) and sphingomyelin phosphodiesterase 1 (SMPD1) (Bi et al., 2021) were identified as two important enzymatic regulators of plasma membrane reorganization in GBM. LPCAT1 seems to control membrane PL saturation (Bi et al., 2019), whereas SMPD1 converts sphingomyelin to ceramide and is crucial for lipid raft composition and clustering of membrane signaling molecules, such as EGFR (Bi et al., 2021). Targeting of both LPCAT1 and SMPD1 has proven beneficial for controlling GBM growth (Bi et al., 2021; Bi et al., 2019), especially in combination with TMZ (Bi et al., 2021).

Taken together, the current body of work on GBM lipid metabolism reveals a beneficial interplay between lipid, cholesterol, and glucose metabolism that seems to support prosurvival metabolic plasticity in GBM. However, many questions remain regarding the molecular drivers of such plasticity and the impact of microenvironmental conditions and therapies. For example, it seems that in the presence of exogenous fatty acids, GBM cells upregulate their glucose consumption (Taib et al., 2019), but the mechanisms remain unclear.

#### *Lipid metabolism modifies the response to anticancer therapies*

There is evidence that lipid metabolism in GBM may modify the response to anticancer therapies (Caragher et al.,

2020; Gupta et al., 2020). Studies performed in GBM, and other tumor models, point to a role for lipid droplets in protecting cancer cells from oxidative stress-induced lipid peroxidation (Jarc and Petan, 2019; Shyu et al., 2018) and nutrient/hypoxic stress (Bensaad et al., 2014; Jarc and Petan, 2019; Jarc et al., 2018) by buffering and delaying the release of lipids. ROS can cause lipid peroxidation of polyunsaturated fatty acids, which will become unstable and break down into oxidative stress second messengers (Barrera, 2012; Dalleau et al., 2013). Ultimately, lipid peroxidation negatively affects the integrity of cellular membranes (Barrera, 2012).

Under hypoxia-induced oxidative stress conditions, inhibition of lipid droplet synthesis increases ROS levels, whereas addition of free fatty acids decreases ROS levels (Bensaad et al., 2014), suggesting that lipid droplets may maintain cellular redox homeostasis under stress conditions. It remains unclear if therapy-induced oxidative stress increases the number of lipid droplets in GBM cells. However, one study shows that RT increases lipid droplet levels, and a higher number is associated with increased radiation resistance in glioma (Tirinato et al., 2021). Hydrolysis of lipid droplets can also provide the energy needed to survive therapy. Therapy-induced increase in autophagy is recognized in most cancers, including in GBM (Tsai et al., 2021; Zois and Koukourakis, 2009) and lipophagy can hydrolyze lipid droplets (Wu et al., 2020).

A hypothesis emerging from these observations is that therapy-induced increase in autophagy/lipophagy may enhance lipid droplet hydrolysis, providing the tumor cells with an alternative way to maintain redox homeostasis and with the energy needed to survive therapy.

GSCs are often associated with increased therapy-resistance and invasion (Bao et al., 2006; Beier et al., 2011) and are characterized by a distinct metabolic phenotype and enhanced metabolic plasticity (Caragher et al., 2020; Hoang-Minh et al., 2018; Vlashi et al., 2011). There is evidence that slow cycling GSCs have higher lipid levels and are enriched in lipid droplets, compared with fast cycling cells, thanks to a basal increase in fatty acid uptake, and this is accompanied by increased resistance to TMZ (Hoang-Minh et al., 2018). Under stress conditions such as low glucose, the slow cycling GSCs break down (by autophagy) their lipid droplets as a source of energy. Targeting fatty acid uptake *via* inhibition of fatty acid binding protein 7 (FABP7) sensitizes slow cycling GSCs to glucose deprivation or glycolysis inhibition (Hoang-Minh et al., 2018).

It is unclear, however, if TMZ treatment may induce such a dependency on lipid droplets and lipid catabolism, although there is evidence that TMZ treatment increases fatty acid uptake in GBM, with a concomitant decrease in glucose uptake *in vitro* and *in vivo* (Caragher et al., 2020). This effect is enhanced in the GSC population and is accompanied by a higher oxygen consumption rate and respiratory capacity that seems to be supported by endogenous fatty acids (Caragher et al., 2020). The TMZ-treated cells also increase the expression of genes involved in fatty acid uptake and  $\beta$ -oxidation (Caragher et al., 2020).

While fatty acid metabolism plays a role in TMZ resistance in GBM, it seems that cholesterol metabolism is preferentially involved in the response to RT. The expression of the master regulator of cholesterol metabolism, SREBP2, and its target genes are upregulated in irradiated GBM cells, and this

response is further enhanced when RT is combined with a dopamine receptor (DR) antagonist (Bhat et al., 2021). Although the mechanisms remain unknown, treating GBM with a combination of RT and DR-antagonists induces specific metabolic rewiring in the form of enhanced cholesterol metabolism, likely aimed at promoting survival under these stressors. Inhibiting cholesterol metabolism, *via* atorvastatin, further reduces GBM survival, pointing to an acquired dependence on cholesterol metabolism after RT/DR-antagonist treatment (Bhat et al., 2021).

This study illustrates that targeting metabolic plasticity has the potential to enhance the therapeutic efficacy, although further investigations are needed to fully appreciate the importance of lipid metabolism in GBM and the contribution to therapy resistance.

### Combating Therapy Resistance by Dismantling GBM's Metabolic Back-Up Plans

Given that glucose is also an essential nutrient for normal cells, especially the brain (Mergenthaler et al., 2013), denying glucose to tumors does not provide an optimal therapeutic window, although attempts have been made in GBM using the glucose competitor, 2-deoxyglucose (2DG), in combination with RT (Singh et al., 2005). While the cancer's greed for glucose may not be the ideal therapeutic target, it has proven quite useful for imaging purposes in the form of FDG-positron emission tomography (PET), widely used for cancer detection, diagnosis, and treatment responses (Kostakoglu et al., 2003), including in GBM (Verger and Langen, 2017). Targeting the *flexibility* with which GBM metabolizes glucose, glutamine, or lipids, rather than specific metabolic nodes, by targeting molecular regulators of metabolic plasticity, will likely prove a more effective way to slow GBM growth and/or render GBM more responsive to current therapies.

#### Targeting glycolytic and redox plasticity

Although hyperactive EGFR signaling instructs GBM cells to grow, this would be futile without sufficient metabolite pools to support growth. Therefore, a more coordinated effort to increase metabolite supply must also take place and some of these efforts are directly regulated by growth signaling. Enhanced levels of metabolite supply can generally be achieved in four ways: increase of exogenous uptake (*i.e.*, glucose), increase of *de novo* synthesis (*i.e.*, SBP), salvage from other macromolecules (*i.e.*, autophagy), or creation of bottlenecks in metabolic fluxes to boost a specific metabolite pool. One example of the latter is the suppression of the enzymatic activity of PKM2, which can be achieved by growth factor signaling (Hitosugi et al., 2009; Li et al., 2016a; Yang et al., 2011) and various posttranslational modifications (Zahra et al., 2020).

This bottleneck in glycolytic flux results in upstream metabolites becoming available for use in glycolytic branches, that is, PPP and SBP, both of which generate macromolecule precursors as well as reducing equivalents in the form of NADPH and GSH (Fig. 2). "Turning off" PKM2 enzymatic activity, therefore, is one flexible way to couple oncogenic growth signaling with metabolic resources necessary to carry out instructions for rapid division and growth or ramp up antioxidant production and precursors for DNA

repair under oxidative stress conditions, such as during RT (Fig. 2). In contrast, forcing PKM2 in the "on" position would deplete the upstream metabolites and prevent the increase in PPP and SBP flux resulting in insufficient amounts of nucleotides and NADPH and, consequently, insufficient GSH.

This would hamper the cell's ability to fight oxidative stress and repair DNA damage, offering a potential cancer-specific target, especially in the context of RT. This has motivated the development of small-molecule activators for PKM2 that lock the enzyme in a high-activity conformation that has shown preclinical therapeutic promise in different tumor types (Anastasiou et al., 2012; Li et al., 2018; Parnell et al., 2013). This approach seems to also slow down GBM growth, however, it has not yet been tested in orthotopic models of GBM (Ding et al., 2020; Guo et al., 2019). Newer generations of PKM2 activators also prevent PKM2 from entering the nucleus, thus further interfering with its moonlighting functions that could intensify therapy resistance (Ding et al., 2020).

Although there is evidence that some PKM2 activators cross the BBB as shown with PET radiotracer analogs of PKM2 activators (Witney et al., 2015), the successful use of such activators for treating GBM would require brain accumulation at therapeutic levels and this remains to be demonstrated. However, even if a brain penetrant PKM2 activator were to be developed, it seems unlikely that it will suppress GBM growth as a single agent, as the metabolically plastic GBM tumors would likely rewire metabolic fluxes to circumvent the blockage in the "PKM2 switch." Combination with other therapies that exploit the metabolic vulnerabilities induced by PKM2 activation would be expected to enhance the effectiveness of this approach. For example, we have shown that combining PKM2 activators with RT can radiosensitize human breast cancers (Zhang et al., 2019), suggesting that this might be a feasible approach in treating GBM if PKM2 activators can effectively accumulate in intracranial GBM.

PGK1 represents another attractive metabolic target in GBM (Qian et al., 2019) for interfering with prosurvival metabolic rewiring. Radioresistant GBM cells display an increased expression of PGK1 (Ding et al., 2014), pointing to a radioprotective role. It is reasonable to postulate that increasing the precursors for the SBP protects GBM from RT-induced cytotoxicity, likely by generating NADPH, and precursors for GSH, as well as providing precursors for nucleotide biosynthesis promoting DNA repair (Fig. 2). A few mechanisms of PGK1 regulation have been identified, including posttranslational modifications [acetylation (Hu et al., 2017), O-GlcNAcylation (Nie et al., 2020), phosphorylation (Qian et al., 2019)], HIF1 regulation (Kress et al., 1998), and EGFR signaling (Li et al., 2016b). The common occurrence of hypoxia and aberrant EGFR signaling in GBM tumors argue in favor of a therapeutic potential for PGK1 targeting.

In addition, evidence that PTEN levels inversely correlate with PGK1 activation (Qian et al., 2019) suggest that targeting PGK1 may be more relevant in the context of a PTEN deletion. Studies have shown that loss of PTEN is associated with increased radioresistance (Pappas et al., 2007; Zhang et al., 2011), including in GBM (Zhang et al., 2011), suggesting a potential therapeutic opportunity in targeting PGK1

in combination with RT in PTEN-negative GBM tumors. It is worth mentioning that while PGK1 targeting would deplete the SBP from its precursor 3PG, GBM cells may still import extracellular serine and glycine (Fig. 2), which might contribute to resistance to this approach. In fact, although not performed in GBM, forced activation of PKM2, which would also divert 3PG away from the SBP has been shown to render cancer cells serine auxotroph (Kung et al., 2012).

Such a possible outcome could be counteracted by implementing a serine/glycine-depleted diet. Serine depletion has been tested *in vivo*, in a colorectal cancer model, without apparent systemic toxicity (Maddocks et al., 2017; Maddocks et al., 2013), but this remains to be tested in combination with RT in GBM.

Another node in the SBP with targeting potential is PHGDH, the enzyme that initiates serine biosynthesis. Engel et al. (2020) have tested the effects of PHGDH inhibition in GBM cells *in vitro* and found that it reduced proliferation and resistance to hypoxia. In other tumor types, PHGDH targeting has been suggested as a way to sensitize cancer cells to chemotherapy (Rathore et al., 2020). The amino acid transporter SLC7A11/xCT constitutes another promising target as its inhibition will prevent import of cysteine, thereby affecting GSH synthesis in cells (Chung et al., 2005; Cobler et al., 2018; Muir et al., 2017). In GBM, inhibition of xCT prevents tumor growth (Chung et al., 2005) and increases sensitivity to TMZ and oxidative stress (Polewski et al., 2016).

However, oxidative stress was induced by hydrogen peroxide treatment rather than radiation, and radiation-specific studies are needed, although there is evidence that in breast cancer, xCT inhibition has a radiosensitizing effect (Cobler et al., 2018). In an interesting observation, Polewski et al. (2016) show that GBM cells increase GSH levels in response to oxidative stress, but this increase is greater when xCT is overexpressed, suggesting a potential resistance mechanism to oxidative stress. Inhibition of xCT as a therapeutic approach would prevent the export of glutamate in the extracellular compartment, leading to a buildup of glutamate inside the cell. As high levels of extracellular glutamate are neurotoxic, inhibition of xCT could also help alleviate the neurologic symptoms of GBM (de Groot and Sontheimer, 2011).

#### Targeting lipid metabolism

Evidence of lipid metabolism reprogramming in GBM basally and following therapies suggests that targeted interventions would benefit GBM patients. Preclinical investigations (Jiang et al., 2014; Zhu et al., 2019) and clinical trials (Altwaigi et al., 2021; Xie et al., 2020) have explored the therapeutic potential of targeting the mevalonate/cholesterol pathway. Statins, which are cholesterol-lowering drugs, block the synthesis of mevalonate by inhibiting HMGCR, the rate-limiting enzyme in the pathway, and are being investigated for their anticancer activity (Di Bello et al., 2020). All nine FDA-approved statins tested *in vitro* with GBM cells inhibited cell proliferation (Jiang et al., 2014; Zhu et al., 2019), with pitavastatin, the most effective, also inhibiting GBM growth *in vivo*, as a single agent (Jiang et al., 2014). Lovastatin and simvastatin increase TMZ-induced apoptosis and impair autophagic flux in GBM cells, although *in vitro*

(Shojaei et al., 2020; Zhu et al., 2019) with additional pre-clinical investigations required to further assess the therapeutic potential.

To the best of our knowledge, one clinical trial has been conducted in GBM to assess the therapeutic potential of statins in combination with RT/chemotherapy. Atorvastatin showed no improvement of progression-free survival or OS at 6 months in GBM, in combination with standard therapy (RT and TMZ), compared with standard therapy alone (Altwaigi et al., 2021), however, newer evidence suggests that it might be beneficial in combination with other novel therapies (Bhat et al., 2021).

Therapeutic targeting of cholesterol metabolism may also be achieved by interfering with its regulation. An interesting target is LXR, a regulator of cholesterol transport. Under normal conditions, LXRs are activated by oxidized forms of cholesterol, named oxysterols. LXR activation upregulates the E3 ligase IDOL, which triggers the ubiquitination and degradation of LDLR. The LDLR family is essential for importing extracellular cholesterol into the cell. Therefore, LXR activation ultimately decreases cholesterol import and intracellular levels. GBM cells do not produce enough oxysterols. This results in inactive LXR and increased import of cholesterol. It follows therefore that forced activation of LXR even in the absence of oxysterols, *via* LXR agonists, may be beneficial in depleting cholesterol from GBM cells and inhibits growth (Guo et al., 2011; Mita et al., 2018; Nguyen et al., 2019; Villa et al., 2016).

The effect of such agonists in preclinical models of GBM seems to vary, with one study using orthotopic GBM tumors and the brain-penetrant LXR agonist, LXR-623, enhancing GBM cell death and significantly increasing survival when used on its own (Villa et al., 2016). In a different GBM model, LXR-623 alone did not significantly affect tumor growth (Nguyen et al., 2019). However, in a subcutaneous GBM model, it did significantly inhibit tumor growth when combined with B cell lymphoma-extra large (BCL-xL) inhibition (Nguyen et al., 2019), suggesting that combination therapies may be required. Another more potent LXR agonist, RGX-104, currently in a phase 1 clinical trial in patients with lymphoma or advanced solid tumors (Clinicaltrials.gov, NCT02922764), has shown promise in various preclinical tumor models, including GBM (Tavazoie et al., 2018).

Given the known dependency of GBM cells to glucose metabolism, there are many ongoing investigations on the therapeutic potential of a ketogenic diet for GBM patients. Ketogenic diets are based on low carbohydrates and high-fat uptake and have been used as a therapy for epilepsy (Simeone et al., 2017). The rationale behind its use in GBM is that while neurons and glial cells use glucose as a primary energy source they can also switch to fatty acid use under glucose deprivation (Panov et al., 2014). Since GBM cells are glucose-addicted, they would suffer from a glucose-restricted diet. However, a multitude of studies now indicate that GBM cells can use fatty acids as an energy source (Guo et al., 2013; Shakya et al., 2021), and that under nutrient stress, they may hydrolyze lipid droplets and use their fatty acid content (Jarc and Petan, 2019).

A recent study demonstrated that the ketogenic diet, on its own, did not slow GBM growth, whereas the inhibition of the rate-limiting enzyme in fatty acid oxidation, carnitine palmitoyltransferase 1A (CPT1A), did (Sperry et al., 2020). This



study suggests that it is very likely that GBM cells will be able to switch to fatty acid oxidation as their energy source in replacement of glucose. Another study demonstrated that lipid loading in GBM leads to decreased survival, by potentiating the paracrine activation of stromal cells, such as macrophages, and angiogenesis (Offer et al., 2019). Clearly, more investigations are needed on the therapeutic potential of the ketogenic diet in combination with standard therapies, or fatty acid oxidation inhibition (Sperry et al., 2020).

### Summary

The current knowledge from published studies strongly supports the notion that robust metabolic plasticity can act as a (flexible) shield against the cytotoxic effects of standard GBM therapies, thus driving therapy resistance. Relevant to RT, two recent studies using personalized genome-scale metabolic flux models to determine the role that tumor metabolism plays in RT resistance across different tumor types, including GBM, identified tumor redox metabolism as a major predictor for RT resistance (Lewis and Kemp, 2021; Lewis et al., 2021). It was demonstrated that radioresistant tumors reroute metabolic fluxes to boost the levels of reducing factors of the cell, most notably NADPH and GSH, thus enhancing clearance of ROS that are generated during RT (Lewis et al., 2021).

Conceptually, these studies posit that the demand for antioxidants to neutralize radiation-induced ROS could outstrip the existing cellular supplies of tumors and that radioresistant tumors compensate by rerouting metabolic fluxes to efficiently fuel antioxidant pathways. Such appreciation also reveals an Achilles' heel, opening up the possibility that interventions designed to target regulators of "metabolic plasticity," rather than specific metabolic pathways, have the potential to improve the therapeutic outcomes in GBM (van Gisbergen et al., 2021). Since metabolic plasticity is made possible by the redundancy in metabolic pathways, such an approach might be even more effective in the context of loss of isozyme diversity (LID).

An exciting recent study by Marczyk et al. (2022) systematically analyzed isozymes affected by cancer-specific LID (relative to a corresponding normal tissue) across a broad range of tumor types and demonstrated that LID may have unsalvageable effects on the redundancy of cancer cell metabolism. Although GBM was not included in the study, LID seems to affect ~5%–30% of tumor isozymes (Marczyk et al., 2022). If LID exists in GBM metabolic enzymes and their intersection with therapy-induced metabolic rewiring is understood, targeting pathways regulated by enzymes with LID would present a promising strategy for creating lethal bottlenecks in metabolic plasticity.

We pose therefore that identifying and appropriately targeting the molecular regulators of metabolic plasticity in GBM to force "metabolic rigidity," especially as it applies to radiation response with regard to maintaining redox homeostasis and properly and swiftly repairing potentially lethal DNA damage, hold promise for further improving this already effective form of therapy in GBM and by extension, patients' outcomes.

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### Authors' Contributions

Conceptualization, writing, editing, figure design, and preparation: J.B. and E.V. Funding acquisition: E.V.

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### References

- Altwaairgi AK, Alghareeb WA, AlNajjar FH, et al. Atorvastatin in combination with radiotherapy and temozolomide for glioblastoma: A prospective phase II study. *Invest New Drugs* 2021;39(1):226–231; doi: 10.1007/s10637-020-00992-5
- Anastasiou D, Poulogiannis G, Asara JM, et al. Inhibition of pyruvate kinase M2 by reactive oxygen species contributes to cellular antioxidant responses. *Science* 2011;334(6060):1278–1283; doi: 10.1126/science.1211485
- Anastasiou D, Yu Y, Israelsen WJ, et al. Pyruvate kinase M2 activators promote tetramer formation and suppress tumorigenesis. *Nat Chem Biol* 2012;8(10):839–847; doi: 10.1038/nchembio.1060
- Babic I, Anderson ES, Tanaka K, et al. EGFR mutation-induced alternative splicing of Max contributes to growth of glycolytic tumors in brain cancer. *Cell Metab* 2013;17(6):1000–1008; doi: 10.1016/j.cmet.2013.04.013
- Bagga P, Behar KL, Mason GF, et al. Characterization of cerebral glutamine uptake from blood in the mouse brain: Implications for metabolic modeling of <sup>13</sup>C NMR data. *J Cereb Blood Flow Metab* 2014;34(10):1666–1672; doi: 10.1038/jcbfm.2014.129
- Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 2006;444(7120):756–760; doi: 10.1038/nature05236
- Barrera G. Oxidative stress and lipid peroxidation products in cancer progression and therapy. *ISRN Oncol* 2012;2012:137289; doi: 10.5402/2012/137289
- Beier D, Schulz JB, Beier CP. Chemoresistance of glioblastoma cancer stem cells—Much more complex than expected. *Mol Cancer* 2011;10:128; doi: 10.1186/1476-4598-10-128
- Beloribi-Djefaffia S, Vasseur S, Guillaumond F. Lipid metabolic reprogramming in cancer cells. *Oncogenesis* 2016;5:e189; doi: 10.1038/oncsis.2015.49
- Bensaad K, Favaro E, Lewis CA, et al. Fatty acid uptake and lipid storage induced by HIF-1 $\alpha$  contribute to cell growth and survival after hypoxia-reoxygenation. *Cell Rep* 2014;9(1):349–365; doi: 10.1016/j.celrep.2014.08.056
- Bensaad K, Tsuruta A, Selak MA, et al. TIGAR, a p53-inducible regulator of glycolysis and apoptosis. *Cell* 2006;126(1):107–120; doi: 10.1016/j.cell.2006.05.036
- Bhat K, Saki M, Cheng F, et al. Dopamine receptor antagonists, radiation, and cholesterol biosynthesis in mouse models of glioblastoma. *J Natl Cancer Inst* 2021;113(8):1094–1104; doi: 10.1093/jnci/djab018
- Bi J, Ichu TA, Zanca C, et al. Oncogene amplification in growth factor signaling pathways renders cancers dependent on membrane lipid remodeling. *Cell Metab* 2019;30(3):525–538 e528; doi: 10.1016/j.cmet.2019.06.014

- Bi J, Khan A, Tang J, et al. Targeting glioblastoma signaling and metabolism with a re-purposed brain-penetrant drug. *Cell Rep* 2021;37(5):109957; doi: 10.1016/j.celrep.2021.109957
- Boado RJ, Black KL, Pardridge WM. Gene expression of GLUT3 and GLUT1 glucose transporters in human brain tumors. *Mol Brain Res* 1994;27(1):51–57; doi: 10.1016/0169-328x(94)90183-x
- Brennan CW, Verhaak RG, McKenna A, et al. The somatic genomic landscape of glioblastoma. *Cell* 2013;155(2):462–477; doi: 10.1016/j.cell.2013.09.034
- Brosnan JT. Interorgan amino acid transport and its regulation. *J Nutr* 2003;133(6 Suppl 1):2068s–2072s; doi: 10.1093/jn/133.6.2068S
- Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 2008;455(7216):1061–1068; doi: 10.1038/nature07385
- Caragher S, Miska J, Shireman J, et al. Temozolomide treatment increases fatty acid uptake in glioblastoma stem cells. *Cancers* 2020;12(11):3126; doi: 10.3390/cancers12113126
- Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2012;2(5):401–404; doi: 10.1158/2159-8290.Cd-12-0095
- Chinnaiyan P, Kensicki E, Bloom G, et al. The metabolomic signature of malignant glioma reflects accelerated anabolic metabolism. *Cancer Res* 2012;72(22):5878–5888; doi: 10.1158/0008-5472.CAN-12-1572-T
- Christofk HR, Vander Heiden MG, Wu N, et al. Pyruvate kinase M2 is a phosphotyrosine-binding protein. *Nature* 2008;452(7184):181–186; doi: 10.1038/nature06667
- Chung WJ, Lyons SA, Nelson GM, et al. Inhibition of cystine uptake disrupts the growth of primary brain tumors. *J Neurosci* 2005;25(31):7101–7110; doi: 10.1523/JNEUROSCI.5258-04.2005
- Cobler L, Zhang H, Suri P, et al. xCT inhibition sensitizes tumors to  $\gamma$ -radiation via glutathione reduction. *Oncotarget* 2018;9(64):32280–32297; doi: 10.18632/oncotarget.25794
- Comelli M, Pretis I, Buso A, et al. Mitochondrial energy metabolism and signalling in human glioblastoma cell lines with different PTEN gene status. *J Bioenerg Biomembr* 2018;50(1):33–52; doi: 10.1007/s10863-017-9737-5
- Cosset E, Ilmjärvi S, Dutoit V, et al. Glut3 addiction is a druggable vulnerability for a molecularly defined subpopulation of glioblastoma. *Cancer Cell* 2017;32(6):856–868 e855; doi: 10.1016/j.ccell.2017.10.016
- da-Silva WS, Gómez-Puyou A, de Gómez-Puyou MT, et al. Mitochondrial bound hexokinase activity as a preventive antioxidant defense: Steady-state ADP formation as a regulatory mechanism of membrane potential and reactive oxygen species generation in mitochondria. *J Biol Chem* 2004;279(38):39846–39855; doi: 10.1074/jbc.M403835200
- Dalleau S, Baradat M, Gueraud F, et al. Cell death and diseases related to oxidative stress: 4-hydroxynonenal (HNE) in the balance. *Cell Death Differ* 2013;20(12):1615–1630; doi: 10.1038/cdd.2013.138
- Dando I, Cordani M, Donadelli M. Mutant p53 and mTOR/PKM2 regulation in cancer cells. *IUBMB Life* 2016;68(9):722–726; doi: 10.1002/iub.1534
- de Groot J, Sontheimer H. Glutamate and the biology of gliomas. *Glia* 2011;59(8):1181–1189; doi: 10.1002/glia.21113
- de Koning TJ, Snell K, Duran M, et al. L-serine in disease and development. *Biochem J* 2003;371(Pt 3):653–661; doi: 10.1042/bj20021785
- De Santis MC, Porporato PE, Martini M, et al. Signaling pathways regulating redox balance in cancer metabolism. *Front Oncol* 2018;8:126; doi: 10.3389/fonc.2018.00126
- DeBerardinis RJ, Chandel NS. We need to talk about the Warburg effect. *Nat Metab* 2020;2(2):127–129; doi: 10.1038/s42255-020-0172-2
- DeBerardinis RJ, Mancuso A, Daikhin E, et al. Beyond aerobic glycolysis: Transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. *Proc Natl Acad Sci U S A* 2007;104(49):19345–19350; doi: 10.1073/pnas.0709747104
- Di Bello E, Zwergel C, Mai A, et al. The innovative potential of statins in cancer: New targets for new therapies. *Front Chem* 2020;8:516; doi: 10.3389/fchem.2020.00516
- Ding H, Cheng YJ, Yan H, et al. Phosphoglycerate kinase 1 promotes radioresistance in U251 human glioma cells. *Oncol Rep* 2014;31(2):894–900; doi: 10.3892/or.2013.2874
- Ding Y, Xue Q, Liu S, et al. Identification of parthenolide dimers as activators of pyruvate kinase M2 in xenograft of glioblastoma multiforme in vivo. *J Med Chem* 2020;63(4):1597–1611; doi: 10.1021/acs.jmedchem.9b01328
- Dittmer D, Pati S, Zambetti G, et al. Gain of function mutations in p53. *Nat Genet* 1993;4(1):42–46; doi: 10.1038/ng0593-42
- Ebert D, Haller RG, Walton ME. Energy contribution of octanoate to intact rat brain metabolism measured by  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy. *J Neurosci* 2003;23(13):5928–5935; doi: 10.1523/jneurosci.23-13-05928.2003
- Engel AL, Lorenz NI, Klann K, et al. Serine-dependent redox homeostasis regulates glioblastoma cell survival. *Br J Cancer* 2020;122(9):1391–1398; doi: 10.1038/s41416-020-0794-x
- Evans SM, Judy KD, Dunphy I, et al. Comparative measurements of hypoxia in human brain tumors using needle electrodes and EF5 binding. *Cancer Res* 2004;64(5):1886–1892; doi: 10.1158/0008-5472.can-03-2424
- Fabian D, Guillermo Prieto Eibl MDP, Alnahhas I, et al. Treatment of glioblastoma (GBM) with the addition of tumor-treating fields (TTF): A review. *Cancers (Basel)* 2019;11(2):174; doi: 10.3390/cancers11020174
- Fan J, Ye J, Kamphorst JJ, et al. Quantitative flux analysis reveals folate-dependent NADPH production. *Nature* 2014;510(7504):298–302; doi: 10.1038/nature13236
- Fang R, Chen X, Zhang S, et al. EGFR/SRC/ERK-stabilized YTHDF2 promotes cholesterol dysregulation and invasive growth of glioblastoma. *Nat Commun* 2021;12(1):177; doi: 10.1038/s41467-020-20379-7
- Filosa S, Fico A, Paglialunga F, et al. Failure to increase glucose consumption through the pentose-phosphate pathway results in the death of glucose-6-phosphate dehydrogenase gene-deleted mouse embryonic stem cells subjected to oxidative stress. *Biochem J* 2003;370(Pt 3):935–943; doi: 10.1042/bj20021614
- Flavahan WA, Wu Q, Hitomi M, et al. Brain tumor initiating cells adapt to restricted nutrition through preferential glucose uptake. *Nat Neurosci* 2013;16(10):1373–1382; doi: 10.1038/nn.3510
- Freed-Pastor WA, Mizuno H, Zhao X, et al. Mutant p53 disrupts mammary tissue architecture via the mevalonate pathway. *Cell* 2012;148(1–2):244–258; doi: 10.1016/j.cell.2011.12.017
- Fu S, Li Z, Xiao L, et al. Glutamine synthetase promotes radiation resistance via facilitating nucleotide metabolism and subsequent DNA damage repair. *Cell Rep* 2019;28(5):1136.e4–1143.e4; doi: 10.1016/j.celrep.2019.07.002

- Gabitova L, Restifo D, Gorin A, et al. Endogenous sterol metabolites regulate growth of EGFR/KRAS-dependent tumors via LXR. *Cell Rep* 2015;12(11):1927–1938; doi: 10.1016/j.celrep.2015.08.023
- Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* 2013;6(269):pl1; doi: 10.1126/scisignal.2004088
- Gao X, Wang H, Yang JJ, et al. Pyruvate kinase M2 regulates gene transcription by acting as a protein kinase. *Mol Cell* 2012;45(5):598–609; doi: 10.1016/j.molcel.2012.01.001
- Geeraerts SL, Heylen E, De Keersmaecker K, et al. The ins and outs of serine and glycine metabolism in cancer. *Nat Metab* 2021;3(2):131–141; doi: 10.1038/s42255-020-00329-9
- Goldman MJ, Craft B, Hastie M, et al. Visualizing and interpreting cancer genomics data via the Xena platform. *Nat Biotechnol* 2020;38(6):675–678; doi: 10.1038/s41587-020-0546-8
- Golias T, Kery M, Radenkovic S, et al. Microenvironmental control of glucose metabolism in tumors by regulation of pyruvate dehydrogenase. *Int J Cancer* 2019;144(4):674–686; doi: 10.1002/ijc.31812
- Guo D, Bell EH, Chakravarti A. Lipid metabolism emerges as a promising target for malignant glioma therapy. *CNS Oncol* 2013;2(3):289–299; doi: 10.2217/cns.13.20
- Guo D, Prins RM, Dang J, et al. EGFR signaling through an Akt-SREBP-1-dependent, rapamycin-resistant pathway sensitizes glioblastomas to antiproliferative therapy. *Sci Signal* 2009;2(101):ra82; doi: 10.1126/scisignal.2000446
- Guo D, Reinitz F, Youssef M, et al. An LXR agonist promotes glioblastoma cell death through inhibition of an EGFR/AKT/SREBP-1/LDLR-dependent pathway. *Cancer Discov* 2011;1(5):442–456; doi: 10.1158/2159-8290.CD-11-0102
- Guo J, Xue Q, Liu K, et al. Dimethylaminomethylolide (DMAMCL) suppresses the proliferation of glioblastoma cells via targeting pyruvate kinase 2 (PKM2) and rewiring aerobic glycolysis. *Front Oncol* 2019;9:993; doi: 10.3389/fonc.2019.00993
- Gupta K, Vuckovic I, Zhang S, et al. Radiation induced metabolic alterations associate with tumor aggressiveness and poor outcome in glioblastoma. *Front Oncol* 2020;10:535; doi: 10.3389/fonc.2020.00535
- Han M, Wang S, Yang N, et al. Therapeutic implications of altered cholesterol homeostasis mediated by loss of CYP46A1 in human glioblastoma. *EMBO Mol Med* 2020;12(1):e10924; doi: 10.15252/emmm.201910924
- Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. *Cell* 2011;144(5):646–674; doi: 10.1016/j.cell.2011.02.013
- Hassanein M, Qian J, Hoeksema MD, et al. Targeting SLC1a5-mediated glutamine dependence in non-small cell lung cancer. *Int J Cancer* 2015;137(7):1587–1597; doi: 10.1002/ijc.29535
- Heneberg P. Redox regulation of hexokinases. *Antioxid Redox Signal* 2019;30(3):415–442; doi: 10.1089/ars.2017.7255
- Hitosugi T, Kang S, Vander Heiden MG, et al. Tyrosine phosphorylation inhibits PKM2 to promote the Warburg effect and tumor growth. *Sci Signal* 2009;2(97):ra73; doi: 10.1126/scisignal.2000431
- Hoang-Minh LB, Siebzehrubel FA, Yang C, et al. Infiltrative and drug-resistant slow-cycling cells support metabolic heterogeneity in glioblastoma. *EMBO J* 2018;37(23); doi: 10.15252/embj.201798772
- Hosios AM, Fiske BP, Gui DY, et al. Lack of evidence for PKM2 protein kinase activity. *Mol Cell* 2015;59(5):850–857; doi: 10.1016/j.molcel.2015.07.013
- Hu H, Zhu W, Qin J, et al. Acetylation of PGK1 promotes liver cancer cell proliferation and tumorigenesis. *Hepatology* 2017;65(2):515–528; doi: 10.1002/hep.28887
- Huang PH, Xu AM, White FM. Oncogenic EGFR signaling networks in glioma. *Sci Signal* 2009;2(87):re6; doi: 10.1126/scisignal.287re6
- Huppertz I, Perez-Perri JJ, Mantas P, et al. Riboregulation of Enolase 1 activity controls glycolysis and embryonic stem cell differentiation. *Mol Cell* 2022;82(14):2666.e11–2680.e11; doi: 10.1016/j.molcel.2022.05.019
- İlhan M. Non-metabolic functions of pyruvate kinase M2: PKM2 in tumorigenesis and therapy resistance. *Neoplasma* 2022;69(4):747–754; doi: 10.4149/neo\_2022\_220119N77
- Irokawa H, Numasaki S, Kato S, et al. Comprehensive analyses of the cysteine thiol oxidation of PKM2 reveal the effects of multiple oxidation on cellular oxidative stress response. *Biochem J* 2021;478(7):1453–1470; doi: 10.1042/bcj20200897
- Jarc E, Kump A, Malavasic P, et al. Lipid droplets induced by secreted phospholipase A2 and unsaturated fatty acids protect breast cancer cells from nutrient and lipotoxic stress. *Biochim Biophys Acta Mol Cell Biol Lipids* 2018;1863(3):247–265; doi: 10.1016/j.bbalip.2017.12.006
- Jarc E, Petan T. Lipid droplets and the management of cellular stress. *Yale J Biol Med* 2019;92(3):435–452.
- Jiang P, Mukthavaram R, Chao Y, et al. In vitro and in vivo anticancer effects of mevalonate pathway modulation on human cancer cells. *Br J Cancer* 2014;111(8):1562–1571; doi: 10.1038/bjc.2014.431
- Jyotsana N, Ta KT, DelGiorno KE. The role of cystine/glutamate antiporter SLC7A11/xCT in the pathophysiology of cancer. *Front Oncol* 2022;12:858462; doi: 10.3389/fonc.2022.858462
- Kant S, Kesarwani P, Prabhu A, et al. Enhanced fatty acid oxidation provides glioblastoma cells metabolic plasticity to accommodate to its dynamic nutrient microenvironment. *Cell Death Dis* 2020;11(4):253; doi: 10.1038/s41419-020-2449-5
- Kim D, Fiske BP, Birsoy K, et al. SHMT2 drives glioma cell survival in ischaemia but imposes a dependence on glycine clearance. *Nature* 2015;520(7547):363–367; doi: 10.1038/nature14363
- Kim JH, Nam B, Choi YJ, et al. Enhanced glycolysis supports cell survival in EGFR-mutant lung adenocarcinoma by inhibiting autophagy-mediated EGFR degradation. *Cancer Res* 2018;78(16):4482–4496; doi: 10.1158/0008-5472.CAN-18-0117
- Kostakoglu L, Agress H, Jr., Goldsmith SJ. Clinical role of FDG PET in evaluation of cancer patients. *Radiographics* 2003;23(2):315–340; quiz 533; doi: 10.1148/rg.232025705
- Kress S, Stein A, Maurer P, et al. Expression of hypoxia-inducible genes in tumor cells. *J Cancer Res Clin Oncol* 1998;124(6):315–320; doi: 10.1007/s004320050175
- Kuehne A, Emmert H, Soehle J, et al. Acute activation of oxidative pentose phosphate pathway as first-line response to oxidative stress in human skin cells. *Mol Cell* 2015;59(3):359–371; doi: 10.1016/j.molcel.2015.06.017
- Kung C, Hixon J, Choe S, et al. Small molecule activation of PKM2 in cancer cells induces serine auxotrophy. *Chem Biol* 2012;19(9):1187–1198; doi: 10.1016/j.chembiol.2012.07.021
- Laezza C, D'Alessandro A, Di Croce L, et al. p53 Regulates the mevalonate pathway in human glioblastoma multiforme. *Cell Death Dis* 2015;6:e1909; doi: 10.1038/cddis.2015.279
- Lee J, Kim HK, Han YM, et al. Pyruvate kinase isozyme type M2 (PKM2) interacts and cooperates with Oct-4 in regulating transcription. *Int J Biochem Cell Biol* 2008;40(5):1043–1054; doi: 10.1016/j.biocel.2007.11.009

- Lewis JE, Forshaw TE, Boothman DA, et al. Personalized genome-scale metabolic models identify targets of redox metabolism in radiation-resistant tumors. *Cell Syst* 2021; 12(1):68.e11–81.e11; doi: 10.1016/j.cels.2020.12.001
- Lewis JE, Kemp ML. Integration of machine learning and genome-scale metabolic modeling identifies multi-omics biomarkers for radiation resistance. *Nat Commun* 2021; 12(1):2700; doi: 10.1038/s41467-021-22989-1
- Li G, Wu J, Li L, et al. p53 Deficiency induces MTHFD2 transcription to promote cell proliferation and restrain DNA damage. *Proc Natl Acad Sci U S A* 2021;118(28):1–12; doi: 10.1073/pnas.2019822118
- Li J, Li S, Guo J, et al. Natural product micheliolide (MCL) irreversibly activates pyruvate kinase M2 and suppresses leukemia. *J Med Chem* 2018;61(9):4155–4164; doi: 10.1021/acs.jmedchem.8b00241
- Li N, Feng L, Liu H, et al. PARP inhibition suppresses growth of EGFR-mutant cancers by targeting nuclear PKM2. *Cell Rep* 2016a;15(4):843–856; doi: 10.1016/j.celrep.2016.03.070
- Li X, Jiang Y, Meisenhelder J, et al. Mitochondria-translocated PGK1 functions as a protein kinase to coordinate glycolysis and the TCA cycle in tumorigenesis. *Mol Cell* 2016b;61(5):705–719; doi: 10.1016/j.molcel.2016.02.009
- Lin H, Patel S, Affleck VS, et al. Fatty acid oxidation is required for the respiration and proliferation of malignant glioma cells. *Neuro Oncol* 2017;19(1):43–54; doi: 10.1093/neuonc/now128
- Lin YH, Satani N, Hammoudi N, et al. An enolase inhibitor for the targeted treatment of ENO1-deleted cancers. *Nat Metab* 2020;2(12):1413–1426; doi: 10.1038/s42255-020-00313-3
- Louis DN, Perry A, Reifenger G, et al. The 2016 World Health Organization classification of tumors of the central nervous system: A summary. *Acta Neuropathol* 2016;131(6):803–820; doi: 10.1007/s00401-016-1545-1
- Lu Z, Hunter T. Metabolic kinases moonlighting as protein kinases. *Trends Biochem Sci* 2018;43(4):301–310; doi: 10.1016/j.tibs.2018.01.006
- Lunt SY, Vander Heiden MG. Aerobic glycolysis: Meeting the metabolic requirements of cell proliferation. *Annu Rev Cell Dev Biol* 2011;27:441–464; doi: 10.1146/annurev-cellbio-092910-154237
- Luo W, Hu H, Chang R, et al. Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. *Cell* 2011;145(5):732–744; doi: 10.1016/j.cell.2011.03.054
- Maddocks OD, Berkers CR, Mason SM, et al. Serine starvation induces stress and p53-dependent metabolic remodelling in cancer cells. *Nature* 2013;493(7433):542–546; doi: 10.1038/nature11743
- Maddocks ODK, Athineos D, Cheung EC, et al. Modulating the therapeutic response of tumours to dietary serine and glycine starvation. *Nature* 2017;544(7650):372–376; doi: 10.1038/nature22056
- Mai WX, Gosa L, Daniels VW, et al. Cytoplasmic p53 couples oncogene-driven glucose metabolism to apoptosis and is a therapeutic target in glioblastoma. *Nat Med* 2017;23(11):1342–1351; doi: 10.1038/nm.4418
- Makinoshima H, Takita M, Matsumoto S, et al. Epidermal growth factor receptor (EGFR) signaling regulates global metabolic pathways in EGFR-mutated lung adenocarcinoma. *J Biol Chem* 2014;289(30):20813–20823; doi: 10.1074/jbc.M114.575464
- Marczyk M, Gunasekharan V, Casadevall D, et al. Comprehensive analysis of metabolic isozyme targets in cancer. *Cancer Res* 2022;82(9):1698–1711; doi: 10.1158/0008-5472.CAN-21-3983
- Marin-Valencia I, Yang C, Mashimo T, et al. Analysis of tumor metabolism reveals mitochondrial glucose oxidation in genetically diverse human glioblastomas in the mouse brain in vivo. *Cell Metab* 2012;15(6):827–837; doi: 10.1016/j.cmet.2012.05.001
- Martin MG, Pfrieger F, Dotti CG. Cholesterol in brain disease: Sometimes determinant and frequently implicated. *EMBO Rep* 2014;15(10):1036–1052; doi: 10.15252/embr.201439225
- Mathupala SP, Heese C, Pedersen PL. Glucose catabolism in cancer cells. The type II hexokinase promoter contains functionally active response elements for the tumor suppressor p53. *J Biol Chem* 1997;272(36):22776–22780; doi: 10.1074/jbc.272.36.22776
- Maugard M, Vigneron PA, Bolanos JP, et al. L-Serine links metabolism with neurotransmission. *Prog Neurobiol* 2021; 197:101896; doi: 10.1016/j.pneurobio.2020.101896
- Mellinghoff IK, Wang MY, Vivanco I, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med* 2005;353(19):2012–2024; doi: 10.1056/NEJMoa051918
- Mergenthaler P, Lindauer U, Dienel GA, et al. Sugar for the brain: The role of glucose in physiological and pathological brain function. *Trends Neurosci* 2013;36(10):587–597; doi: 10.1016/j.tins.2013.07.001
- Mita MM, Mita AC, Chmielowski B, et al. Pharmacodynamic and clinical activity of RGX-104, a first-in-class immunotherapy targeting the liver-X nuclear hormone receptor (LXR), in patients with refractory malignancies. *J Clin Oncol* 2018;36(15 Suppl):3095–3095; doi: 10.1200/JCO.2018.36.15\_suppl.3095
- Morrow D, Minami J, Nathanson DA. Metabolic vulnerabilities in brain cancer. *Neurosurg Clin N Am* 2021;32(2):159–169; doi: 10.1016/j.nec.2020.12.006
- Moutinho M, Nunes MJ, Rodrigues E. Cholesterol 24-hydroxylase: Brain cholesterol metabolism and beyond. *Biochim Biophys Acta* 2016;1861(12 Pt A):1911–1920; doi: 10.1016/j.bbaplip.2016.09.011
- Muir A, Danai LV, Gui DY, et al. Environmental cystine drives glutamine anaplerosis and sensitizes cancer cells to glutaminase inhibition. *Elife* 2017;6:e27713; doi: 10.7554/eLife.27713
- Mukherjee J, Phillips JJ, Zheng S, et al. Pyruvate kinase M2 expression, but not pyruvate kinase activity, is up-regulated in a grade-specific manner in human glioma. *PLoS One* 2013; 8(2):e57610; doi: 10.1371/journal.pone.0057610
- Nakamizo S, Sasayama T, Shinohara M, et al. GC/MS-based metabolomic analysis of cerebrospinal fluid (CSF) from glioma patients. *J Neurooncol* 2013;113(1):65–74; doi: 10.1007/s11060-013-1090-x
- Nguyen TTT, Ishida CT, Shang E, et al. Activation of LXRbeta inhibits tumor respiration and is synthetically lethal with BCL-xL inhibition. *EMBO Mol Med* 2019;11(10):e10769; doi: 10.15252/emmm.201910769
- Nie H, Ju H, Fan J, et al. O-GlcNAcylation of PGK1 coordinates glycolysis and TCA cycle to promote tumor growth. *Nat Commun* 2020;11(1):36; doi: 10.1038/s41467-019-13601-8
- Obara-Michlewska M, Szeliga M. Targeting glutamine addiction in gliomas. *Cancers (Basel)* 2020;12(2):310; doi: 10.3390/cancers12020310
- Offer S, Menard JA, Perez JE, et al. Extracellular lipid loading augments hypoxic paracrine signaling and promotes glioma

- angiogenesis and macrophage infiltration. *J Exp Clin Cancer Res* 2019;38(1):241; doi: 10.1186/s13046-019-1228-6
- Oh S, Yeom J, Cho HJ, et al. Integrated pharmacoproteogenomics defines two subgroups in isocitrate dehydrogenase wild-type glioblastoma with prognostic and therapeutic opportunities. *Nat Commun* 2020;11(1):3288; doi: 10.1038/s41467-020-17139-y
- Oizel K, Chauvin C, Oliver L, et al. Efficient mitochondrial glutamine targeting prevails over glioblastoma metabolic plasticity. *Clin Cancer Res* 2017;23(20):6292–6304; doi: 10.1158/1078-0432.CCR-16-3102
- Oizel K, Yang C, Renoult O, et al. Glutamine uptake and utilization of human mesenchymal glioblastoma in orthotopic mouse model. *Cancer Metab* 2020;8:9; doi: 10.1186/s40170-020-00215-8.
- Olzmann JA, Carvalho P. Dynamics and functions of lipid droplets. *Nat Rev Mol Cell Biol* 2019;20(3):137–155; doi: 10.1038/s41580-018-0085-z.
- Owen OE, Kalhan SC, Hanson RW. The key role of anaplerosis and cataplerosis for citric acid cycle function. *J Biol Chem* 2002;277(34):30409–30412; doi: 10.1074/jbc.R200006200
- Panov A, Orynbayeva Z, Vavilin V, et al. Fatty acids in energy metabolism of the central nervous system. *Biomed Res Int* 2014;2014:472459; doi: 10.1155/2014/472459
- Pappas G, Zumstein LA, Munshi A, et al. Adenoviral-mediated PTEN expression radiosensitizes non-small cell lung cancer cells by suppressing DNA repair capacity. *Cancer Gene Ther* 2007;14(6):543–549; doi: 10.1038/sj.cgt.7701050
- Parnell KM, Foulks JM, Nix RN, et al. Pharmacologic activation of PKM2 slows lung tumor xenograft growth. *Mol Cancer Ther* 2013;12(8):1453–1460; doi: 10.1158/1535-7163.Mct-13-0026
- Patra KC, Hay N. The pentose phosphate pathway and cancer. *Trends Biochem Sci* 2014;39(8):347–354; doi: 10.1016/j.tibs.2014.06.005
- Pavlova NN, Thompson CB. The emerging hallmarks of cancer metabolism. *Cell Metab* 2016;23(1):27–47; doi: 10.1016/j.cmet.2015.12.006
- Perelroizen R, Philosofof B, Budick-Harmelin N, et al. Astrocyte immunometabolic regulation of the tumour microenvironment drives glioblastoma pathogenicity. *Brain* 2022;145(9):3288–3307; doi: 10.1093/brain/awac222
- Pirmoradi L, Seyfizadeh N, Ghavami S, et al. Targeting cholesterol metabolism in glioblastoma: A new therapeutic approach in cancer therapy. *J Investig Med* 2019;67(4):715–719; doi: 10.1136/jim-2018-000962
- Polewski MD, Reveron-Thornton RF, Cherryholmes GA, et al. Increased expression of system xc<sup>-</sup> in glioblastoma confers an altered metabolic state and temozolomide resistance. *Mol Cancer Res* 2016;14(12):1229–1242; doi: 10.1158/1541-7786.MCR-16-0028
- Possemato R, Marks KM, Shaul YD, et al. Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. *Nature* 2011;476(7360):346–350; doi: 10.1038/nature10350
- Qian X, Li X, Shi Z, et al. PTEN suppresses glycolysis by dephosphorylating and inhibiting autophosphorylated PKG1. *Mol Cell* 2019;76(3):516.e7–527.e7; doi: 10.1016/j.molcel.2019.08.006
- Quek LE, van Geldermalsen M, Guan YF, et al. Glutamine addiction promotes glucose oxidation in triple-negative breast cancer. *Oncogene* 2022;41(34):4066–4078; doi: 10.1038/s41388-022-02408-5
- Ralsler M, Wamelink MM, Kowald A, et al. Dynamic rerouting of the carbohydrate flux is key to counteracting oxidative stress. *J Biol* 2007;6(4):10; doi: 10.1186/jbiol61
- Ralsler M, Wamelink MM, Latkolik S, et al. Metabolic reconfiguration precedes transcriptional regulation in the anti-oxidant response. *Nat Biotechnol* 2009;27(7):604–605; doi: 10.1038/nbt0709-604
- Rathore R, Schutt CR, Van Tine BA. PHGDH as a mechanism for resistance in metabolically-driven cancers. *Cancer Drug Resist* 2020;3:762–774; doi: 10.20517/cdr.2020.46
- Read GH, Bailleul J, Vlashi E, et al. Metabolic response to radiation therapy in cancer. *Mol Carcinog* 2022;61(2):200–224; doi: 10.1002/mc.23379
- Sánchez-Castillo A, Vooijs M, Kampen KR. Linking serine/glycine metabolism to radiotherapy resistance. *Cancers* 2021;13(6):1191; doi: 10.3390/cancers13061191
- Scalise M, Pochini L, Console L, et al. The human SLC1A5 (ASCT2) amino acid transporter: From function to structure and role in cell biology. *Front Cell Dev Biol* 2018;6:96; doi: 10.3389/fcell.2018.00096
- Schapiro AHV. *Neurology and Clinical Neuroscience*. Mosby Elsevier: Philadelphia PA, 2007; p. 1336.
- Schwartzberg-Bar-Yoseph F, Armoni M, Karnieli E. The tumor suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4 gene expression. *Cancer Res* 2004;64(7):2627–2633; doi: 10.1158/0008-5472.can-03-0846
- Shakya S, Gromovsky AD, Hale JS, et al. Altered lipid metabolism marks glioblastoma stem and non-stem cells in separate tumor niches. *Acta Neuropathol Commun* 2021;9(1):101; doi: 10.1186/s40478-021-01205-7
- Shojaei S, Koleini N, Samiei E, et al. Simvastatin increases temozolomide-induced cell death by targeting the fusion of autophagosomes and lysosomes. *FEBS J* 2020;287(5):1005–1034; doi: 10.1111/febs.15069
- Shyu P, Jr., Wong XFA, Crasta K, et al. Dropping in on lipid droplets: Insights into cellular stress and cancer. *Biosci Rep* 2018;38(5):1–20; doi: 10.1042/BSR20180764
- Šimčíková D, Gardáš D, Hložková K, et al. Loss of hexokinase 1 sensitizes ovarian cancer to high-dose metformin. *Cancer Metab* 2021;9(1):41; doi: 10.1186/s40170-021-00277-2
- Simeone TA, Matthews SA, Samson KK, et al. Regulation of brain PPAR $\gamma$ 2 contributes to ketogenic diet anti-seizure efficacy. *Exp Neurol* 2017;287(Pt 1):54–64; doi: 10.1016/j.expneurol.2016.08.006
- Singh D, Banerji AK, Dwarakanath BS, et al. Optimizing cancer radiotherapy with 2-deoxy-d-glucose dose escalation studies in patients with glioblastoma multiforme. *Strahlenther Onkol* 2005;181(8):507–514; doi: 10.1007/s00066-005-1320-z
- Sizemore ST, Zhang M, Cho JH, et al. Pyruvate kinase M2 regulates homologous recombination-mediated DNA double-strand break repair. *Cell Res* 2018;28(11):1090–1102; doi: 10.1038/s41422-018-0086-7
- Son J, Lyssiotis CA, Ying H, et al. Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. *Nature* 2013;496(7443):101–105; doi: 10.1038/nature12040
- Song Y, Luo Q, Long H, et al. Alpha-enolase as a potential cancer prognostic marker promotes cell growth, migration, and invasion in glioma. *Mol Cancer* 2014;13:65; doi: 10.1186/1476-4598-13-65
- Sperry J, Condro MC, Guo L, et al. Glioblastoma utilizes fatty acids and ketone bodies for growth allowing progression

- during ketogenic diet therapy. *iScience* 2020;23(9):101453; doi: 10.1016/j.isci.2020.101453
- Spitz DR, Azzam EI, Li JJ, et al. Metabolic oxidation/reduction reactions and cellular responses to ionizing radiation: A unifying concept in stress response biology. *Cancer Metastasis Rev* 2004;23(3–4):311–322; doi: 10.1023/B:CANC.0000031769.14728.bc
- Stanke KM, Wilson C, Kidambi S. High expression of glycolytic genes in clinical glioblastoma patients correlates with lower survival. *Front Mol Biosci* 2021;8:752404; doi: 10.3389/fmolb.2021.752404
- Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-Year analysis of the EORTC-NCIC trial. *Lancet Oncol* 2009;10(5):459–466; doi: 10.1016/s1470-2045(09)70025-7
- Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005;352(10):987–996; doi: 10.1056/NEJMoa043330
- Sullivan JP, Nahed BV, Madden MW, et al. Brain tumor cells in circulation are enriched for mesenchymal gene expression. *Cancer Discov* 2014;4(11):1299–1309; doi: 10.1158/2159-8290.Cd-14-0471
- Sun L, Shukair S, Naik TJ, et al. Glucose phosphorylation and mitochondrial binding are required for the protective effects of hexokinases I and II. *Mol Cell Biol* 2008;28(3):1007–1017; doi: 10.1128/mcb.00224-07
- Sun Q, Chen X, Ma J, et al. Mammalian target of rapamycin up-regulation of pyruvate kinase isoenzyme type M2 is critical for aerobic glycolysis and tumor growth. *Proc Natl Acad Sci U S A* 2011;108(10):4129–4134; doi: 10.1073/pnas.1014769108
- Taib B, Aboussalah AM, Moniruzzaman M, et al. Lipid accumulation and oxidation in glioblastoma multiforme. *Sci Rep* 2019;9(1):19593; doi: 10.1038/s41598-019-55985-z
- Takano T, Lin JH, Arcuino G, et al. Glutamate release promotes growth of malignant gliomas. *Nat Med* 2001;7(9):1010–1015; doi: 10.1038/nm0901-1010
- Tanaka K, Sasayama T, Irino Y, et al. Compensatory glutamine metabolism promotes glioblastoma resistance to mTOR inhibitor treatment. *J Clin Invest* 2015;125(4):1591–1602; doi: 10.1172/JCI78239
- Tang Z, He Z. TIGAR promotes growth, survival and metastasis through oxidation resistance and AKT activation in glioblastoma. *Oncol Lett* 2019;18(3):2509–2517; doi: 10.3892/ol.2019.10574
- Tardito S, Oudin A, Ahmed SU, et al. Glutamine synthetase activity fuels nucleotide biosynthesis and supports growth of glutamine-restricted glioblastoma. *Nat Cell Biol* 2015;17(12):1556–1568; doi: 10.1038/ncb3272
- Tateishi K, Iafraite AJ, Ho Q, et al. Myc-driven glycolysis is a therapeutic target in glioblastoma. *Clin Cancer Res* 2016;22(17):4452–4465; doi: 10.1158/1078-0432.CCR-15-2274
- Tavazoie MF, Pollack I, Tanqueco R, et al. LXR/ApoE activation restricts innate immune suppression in cancer. *Cell* 2018;172(4):825.e18–840.e18; doi: 10.1016/j.cell.2017.12.026
- Tirinato L, Marafioti MG, Pagliari F, et al. Lipid droplets and ferritin heavy chain: A devilish liaison in human cancer cell radioresistance. *Elife* 2021;10:e72943; doi: 10.7554/eLife.72943
- Tsai CY, Ko HJ, Huang CF, et al. Ionizing radiation induces resistant glioblastoma stem-like cells by promoting autophagy via the Wnt/beta-catenin pathway. *Life (Basel)* 2021;11(5):451; doi: 10.3390/life11050451
- van Gisbergen MW, Zwilling E, Dubois LJ. Metabolic rewiring in radiation oncology toward improving the therapeutic ratio. *Front Oncol* 2021;11:653621; doi: 10.3389/fonc.2021.653621
- Verger A, Langen KJ. PET Imaging in Glioblastoma: Use in Clinical Practice. In: *Glioblastoma*. (De Vleeschouwer S. ed.) Codon Publications Copyright: Brisbane, 2017; pp. 155–175.
- Verhaak RG, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell* 2010;17(1):98–110; doi: 10.1016/j.ccr.2009.12.020
- Villa GR, Hulce JJ, Zanca C, et al. An LXR-cholesterol axis creates a metabolic co-dependency for brain cancers. *Cancer Cell* 2016;30(5):683–693; doi: 10.1016/j.ccell.2016.09.008
- Vlashi E, Lagadec C, Vergnes L, et al. Metabolic state of glioma stem cells and nontumorigenic cells. *Proc Natl Acad Sci U S A* 2011;108(38):16062–16067; doi: 10.1073/pnas.1106704108
- Walker MD, Alexander E, Jr., Hunt WE, et al. Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial. *J Neurosurg* 1978;49(3):333–343; doi: 10.3171/jns.1978.49.3.0333
- Walker MD, Strike TA, Sheline GE. An analysis of dose-effect relationship in the radiotherapy of malignant gliomas. *Int J Radiat Oncol Biol Phys* 1979;5(10):1725–1731.
- Wang HJ, Hsieh YJ, Cheng WC, et al. JMJD5 regulates PKM2 nuclear translocation and reprograms HIF-1 $\alpha$ -mediated glucose metabolism. *Proc Natl Acad Sci U S A* 2014;111(1):279–284; doi: 10.1073/pnas.1311249111
- Wang LB, Karpova A, Gritsenko MA, et al. Proteogenomic and metabolomic characterization of human glioblastoma. *Cancer Cell* 2021;39(4):509.e20–528.e20; doi: 10.1016/j.ccell.2021.01.006
- Wang X, Huang Z, Wu Q, et al. MYC-regulated mevalonate metabolism maintains brain tumor-initiating cells. *Cancer Res* 2017;77(18):4947–4960; doi: 10.1158/0008-5472.Can-17-0114
- Warburg O. On the metabolism of carcinoma cells. *Biochem Z* 1924;152(309–344):309.
- Witney TH, James ML, Shen B, et al. PET imaging of tumor glycolysis downstream of hexokinase through noninvasive measurement of pyruvate kinase M2. *Sci Transl Med* 2015;7(310):310.
- Wolf A, Agnihotri S, Micallef J, et al. Hexokinase 2 is a key mediator of aerobic glycolysis and promotes tumor growth in human glioblastoma multiforme. *J Exp Med* 2011;208(2):313–326; doi: 10.1084/jem.20101470
- Wu X, Geng F, Cheng X, et al. Lipid droplets maintain energy homeostasis and glioblastoma growth via autophagic release of stored fatty acids. *iScience* 2020;23(10):101569; doi: 10.1016/j.isci.2020.101569
- Xia L, Wang XR, Wang XL, et al. A novel role for pyruvate kinase M2 as a corepressor for P53 during the DNA damage response in human tumor cells. *J Biol Chem* 2016;291(50):26138–26150; doi: 10.1074/jbc.M116.737056
- Xie Y, Lu Q, Lenahan C, et al. Whether statin use improves the survival of patients with glioblastoma? A meta-analysis. *Medicine (Baltimore)* 2020;99(9):e18997; doi: 10.1097/MD.00000000000018997
- Yan Y, Takayasu T, Hines G, et al. Landscape of genomic alterations in IDH wild-type glioblastoma identifies PI3K as a favorable prognostic factor. *JCO Precis Oncol* 2020;4:575–584; doi: 10.1200/PO.19.00385
- Yang M, Vousden KH. Serine and one-carbon metabolism in cancer. *Nat Rev Cancer* 2016;16(10):650–662; doi: 10.1038/nrc.2016.81

- Yang R, Li X, Wu Y, et al. EGFR activates GDH1 transcription to promote glutamine metabolism through MEK/ERK/ELK1 pathway in glioblastoma. *Oncogene* 2020;39(14):2975–2986; doi: 10.1038/s41388-020-1199-2
- Yang W, Xia Y, Ji H, et al. Nuclear PKM2 regulates beta-catenin transactivation upon EGFR activation. *Nature* 2011; 480(7375):118–122; doi: 10.1038/nature10598
- Yang W, Zheng Y, Xia Y, et al. ERK1/2-dependent phosphorylation and nuclear translocation of PKM2 promotes the Warburg effect. *Nat Cell Biol* 2012;14(12):1295–1304; doi: 10.1038/ncb2629
- Zahra K, Dey T, Ashish, et al. Pyruvate kinase M2 and cancer: The role of PKM2 in promoting tumorigenesis. *Front Oncol* 2020;10:159; doi: 10.3389/fonc.2020.00159
- Zhang C, Kang C, Wang P, et al. MicroRNA-221 and -222 regulate radiation sensitivity by targeting the PTEN pathway. *Int J Radiat Oncol Biol Phys* 2011;80(1):240–248; doi: 10.1016/j.ijrobp.2010.12.049
- Zhang C, Liu J, Liang Y, et al. Tumour-associated mutant p53 drives the Warburg effect. *Nat Commun* 2013;4:2935; doi: 10.1038/ncomms3935.
- Zhang L, Bailleul J, Yazal T, et al. PK-M2-mediated metabolic changes in breast cancer cells induced by ionizing radiation. *Breast Cancer Res Treat* 2019;178(1):75–86; doi: 10.1007/s10549-019-05376-9
- Zhang Y, Dube C, Gibert M, Jr., et al. The p53 pathway in glioblastoma. *Cancers (Basel)* 2018a;10(9):297; doi: 10.3390/cancers10090297
- Zhang Y, Yu G, Chu H, et al. Macrophage-associated PGK1 phosphorylation promotes aerobic glycolysis and tumorigenesis. *Mol Cell* 2018b;71(2):201.e7–215.e7; doi: 10.1016/j.molcel.2018.06.023
- Zhou W, Wahl DR. Metabolic abnormalities in glioblastoma and metabolic strategies to overcome treatment resistance. *Cancers (Basel)* 2019;11(9):1231; doi: 10.3390/cancers11091231
- Zhou W, Yao Y, Scott AJ, et al. Purine metabolism regulates DNA repair and therapy resistance in glioblastoma. *Nat Commun* 2020;11(1):3811; doi: 10.1038/s41467-020-17512-x
- Zhu Z, Zhang P, Li N, et al. Lovastatin enhances cytotoxicity of temozolomide via impairing autophagic flux in glioblastoma cells. *Biomed Res Int* 2019;2019:2710693; doi: 10.1155/2019/2710693
- Zois CE, Koukourakis MI. Radiation-induced autophagy in normal and cancer cells: Towards novel cytoprotection and radio-sensitization policies? *Autophagy* 2009;5(4):442–450; doi: 10.4161/auto.5.4.7667

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### Abbreviations Used

|              |   |
|--------------|---|
| 1,3-PG       | = 1,3-bisphosphoglycerate                             |
| 1,6-FBP      | = fructose-1,6-bisphosphate                           |
| 1CM          | = one-carbon metabolism                               |
| 3PG          | = 3-phosphoglycerate                                  |
| 6PGD         | = 6-phosphogluconate dehydrogenase                    |
| $\alpha$ -KG | = $\alpha$ -ketoglutarate                             |
| ABCA1        | = ATP-binding cassette transporter A1                 |
| Ac-CoA       | = acetyl-CoA  |
| ASCT2        | = Alanine, Serine, Cysteine Transporter 2             |
| BBB          | = blood–brain barrier                                 |
| CNV          | = copy number variation                               |
| CYP46A1      | = cholesterol 24-hydroxylase                          |
| DR           | = dopamine receptor                                   |
| EGFR         | = epidermal growth factor receptor                    |
| EGFR-vIII    | = EGFR variant III                                    |
| ENO          | = enolase   |
| F6P          | = fructose-6-phosphate                                |
| FA           | = fatty acid  |
| FADH2        | = flavin adenine dinucleotide                         |
| FBP          | = fructose bisphosphatase                             |
| FDG          | = fluorodeoxyglucose                                  |
| G6P          | = glucose-6-phosphate                                 |
| G6PD         | = glucose-6-phosphate dehydrogenase                   |
| GAPDH        | = glyceraldehyde-3-phosphate dehydrogenase            |
| GBM          | = glioblastoma  |
| GLS          | = glutaminase   |
| GDH1         | = glutamate dehydrogenase-1                           |
| GLUT         | = glucose transporter                                 |
| GS           | = glutamine synthetase                                |
| GSC          | = GBM stem cell                                       |
| GSH          | = reduced glutathione                                 |
| HIF          | = hypoxia-inducible factor                            |
| HK           | = hexokinase  |
| HMGR         | = 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase    |
| IDH          | = isocitrate dehydrogenase                            |
| IL6          | = interleukin 6                                       |
| LDLR         | = low-density lipoprotein receptor                    |
| LGG          | = low-grade gliomas                                   |
| LID          | = loss of isozyme diversity                           |
| LPCAT1       | = lysophosphatidylcholine acyltransferase 1           |
| LXR          | = liver X receptor                                    |
| mRNA         | = messenger RNA                                       |
| MTHFD        | = methylenetetrahydrofolate dehydrogenase             |
| mTORC1       | = mammalian target of rapamycin complex 1             |
| NADH         | = nicotinamide adenine dinucleotide                   |
| NADPH        | = reduced nicotinamide adenine dinucleotide phosphate |
| OS           | = overall survival                                    |
| PDH          | = pyruvate dehydrogenase complex                      |
| PDK1         | = pyruvate dehydrogenase kinase 1                     |
| PEP          | = phosphoenolpyruvate                                 |
| PET          | = positron emission tomography                        |
| PFK          | = phosphofructokinase                                 |
| PGK1         | = phosphoglycerate kinase 1                           |
| PHGDH        | = phosphoglycerate dehydrogenase                      |
| PKM2         | = M2 isoform of pyruvate kinase                       |
| PL           | = phospholipid  |

**Abbreviations Used (Cont.)**

PPP = pentose phosphate pathway  
PSAT1 = phosphoserine aminotransferase 1  
PSPH = phosphoserine phosphatase  
PTEN = phosphatase and tensin homolog deleted  
on chromosome 10  
R5P = ribose-5-phosphate  
ROS = reactive oxygen species  
RT = radiation therapy  
SBP = serine biosynthesis pathway  
SHMT = serine hydroxymethyl transferase  
SLC2A1 = solute carrier family 2 member 1  
(GLUT1)

SLC2A3 = solute carrier family 2 member 3 (GLUT3)  
SLC7A11/xCT = cystine/glutamate antiporter  
SMPD1 = sphingomyelin phosphodiesterase 1  
SREBP = sterol regulatory element-binding protein  
TAA = tumor-associated astrocyte  
TAG = triacylglycerol  
TCA = tricarboxylic acid  
TCGA = The Cancer Genome Atlas  
TIGAR = TP53-induced glycolysis and apoptosis  
regulator  
TME = tumor microenvironment  
TMZ = temozolomide  
WT = wild-type