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Chapter 4

Brain Tumor Metabolism — Unraveling Its Role in Finding New Therapeutic Targets

Vera Miranda Gonçalves, Fátima Baltazar and Rui Manuel Reis

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1. Introduction

Primary tumors of brain account for approximately 2-3% of all cancers, with annual incidence approximately 15 patients per 100,000 people and the prevalence has been estimated in 69 patients per 100,000 people. Several brain tumor types evolve from glial or neuronal precursors, being the tumors of glial cells the most common and denominated gliomas [1, 2]. Gliomas are histologically classified according to the World Health Organization (WHO) classification into four malignancy grades[3, 4]. Pilocytic astrocytomas (WHO grade I) are benign tumors that can usually be cured after surgical resection. Diffuse astrocytomas (WHO grade II) exhibit a slow growth, but have an inevitable tendency to progress to higher grade lesions, such as anaplastic gliomas (WHO grade III) and glioblastomas (WHO grade IV). Anaplastic gliomas are rapidly growing malignant tumors that, in addition to surgery, require aggressive adjuvant therapy. Glioblastomas (GBMs) are the most malignant and frequent type of gliomas, which are preferentially manifested in aged adults with a peak of incidence between 50-60 years old [4]. Glioblastomas may evolve from lower grade tumors as described and are mentioned secondary glioblastomas, although most of GBMs arise rapidly without the evidence of less malignant lesion, and are denominated *de novo* or primary glioblastomas [2, 4].

The current standard therapy for GBM includes tumor resection followed by radiation and concomitant chemotherapy, with temozolomide being the only approved drug that shows some efficacy in this disease [5]. In the last decade, specific inhibitors of oncogenic signaling pathways such as EGFR, PI3K/Akt, and VEGF have made progress with some of them currently tested in clinical trials. Nowadays, bevacizumab (avastin®), a humanized monoclonal antibody against VEGF is approved as a second line of treatment for recurrent GBMs and is currently in phase III clinical trials for the treatment of initial GBMs [6]. Antiangiogenic

therapy with avastin improved radiographic response and 6 month of progression free survival, however with modest or little effect on overall survival, when in combination with TMZ during and after radiotherapy [7, 8]. Besides, its role in promoting vascular normalization, the effect on tumor cell invasion is still controversial. Avastin treatment induces infiltration in U87 xenograft model and also was associated with diffusing invasive recurrence in some GBM patients [9, 10]. Additionally, it was observed that vasculature normalization with bevacizumab treatment leads to increased hypoxia and consequently acquisition of resistance [11]. Despite progress in new molecular-based therapies, the prognosis of glioblastomas patients is still very dismal [12, 13]. Thus, exploitation of new molecular targets becomes crucial in neuro-oncology.

In recent years, understanding the regulation of tumor metabolism has significantly improved. Accumulating evidence showed that tumor cells reprogram their metabolism to meet high energy demands, coordinate markedly elevated biosynthetic processes and energy production, which in turn promote rapid growth and division of tumor cells [14-17]. Thus, targeting metabolism has become a novel promising strategy for treating cancers, particularly glioblastomas.

2. Tumor metabolism

During cancer progression, molecular changes are associated to metabolic reprogramming [18, 19], which is nowadays defined as a new hallmark of cancer [20]. In mammalian cells, namely quiescent cells or differentiated tissues, glycolysis is reduced in the presence of oxygen and energy production arises from mitochondrial oxidative phosphorylation which oxidizes pyruvate to CO_2 and H_2O, known as "Pasteur effect" (Figure 1) [21]. However, in tumor cells, like proliferating tissues, there is high glycolytic activity even in the presence of oxygen, being glycolysis the major source of energy. This phenomenon is known as "Warburg effect". As a result, tumor cells convert most of the incoming glucose into lactate (around 85 %) rather than metabolizing pyruvate in the mitochondria through oxidative phosphorylation (around 5%) (Figure 1) [16, 21, 22].

2.1. Glycolytic metabolism in brain tumors

As above mentioned, in tumor cells, even in the presence of oxygen, glucose is converted into lactate instead of being oxidized in mitochondria, being glycolysis the major source of energy [16]. It has been described that glioblastomas present metabolic remodeling, increasing glycolytic activity about 3-fold when compared to normal brain tissue [23, 24]. Thus, an increase in several glycolytic enzymes was observed, such as hexokinase II (HKII), pyruvate kinase (PKM), as well as the glucose transporters (GLUTs). Importantly, several studies reported these molecules as important mediators in glycolytic metabolism, constituting attractive molecular targets (Figure 2).

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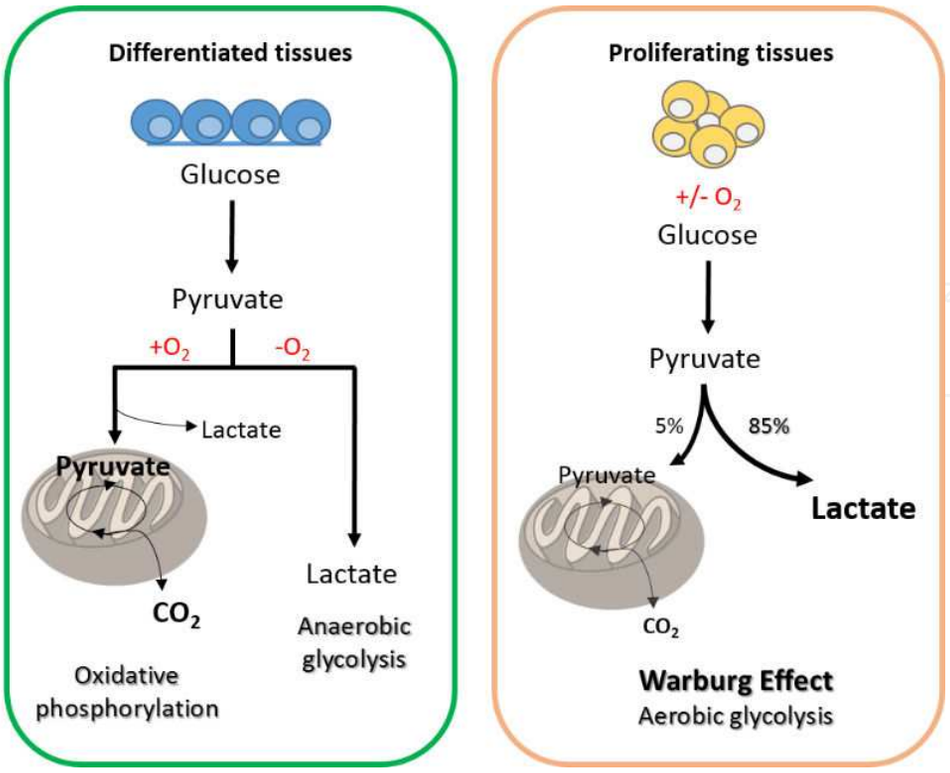

Figure 1. Schematic representation of the metabolic differences between differentiated tissues and proliferating tissues. In the presence of oxygen, non-proliferating tissues metabolize glucose to pyruvate and oxidize it in mitochondria through oxidative phosphorylation. On the other hand, glucose is metabolized to lactate when in the absence of oxygen. In proliferative tissues, like tumor cells, glucose is metabolized to pyruvate and even in the presence of oxygen pryruvate is converted into lactate,a phenomenon denominated aerobic glycolysis or Warburg effect.

2.1.1. Glucose Transporters (GLUTs)

Glucose is the main source of energy in most tissues, including brain. GLUTs are transmembrane transporters that perform the uptake of glucose into the cell. The GLUT family is composed by 12 isoforms, however only GLUT1, GLUT3, and GLUT12 have been described as transporters of glucose [25]. GLUT1 is ubiquitously expressed and it is responsible for providing basal glucose to different tissues and cells. In brain, GLUT1 is expressed in astrocytes, whereas GLUT3 is observed in neurons [26].

In the tumoral context, overexpression of specific isoforms of GLUTs has been reported [27, 28]. Most frequently, an increase in GLUT1 expression has been observed in several solid tumors compared with the corresponding normal tissue [27, 28]. However, it has been verified that their expression is tissue specific and some tumors overexpressed other isoforms, such as GLUT12 in prostate cancer [29]. Concerning brain tumors, few studies have evaluated GLUT expression, where it is described that glioblastomas have an increased expression of GLUT1 and GLUT3 when compared with low grade gliomas and normal brain [30, 31]. In fact, both the isoforms are downstream targets of hypoxia-inducible factor 1α (HIF-1α), a transcription factor that is frequently present in glioblastomas. GLUT1 expression is observed in vessels of the normal brain tissues and presents a focal expression in the perinecrotic regions of GBMs, suggesting that their expression is associated with hypoxic regions in glioblastomas (Miranda-

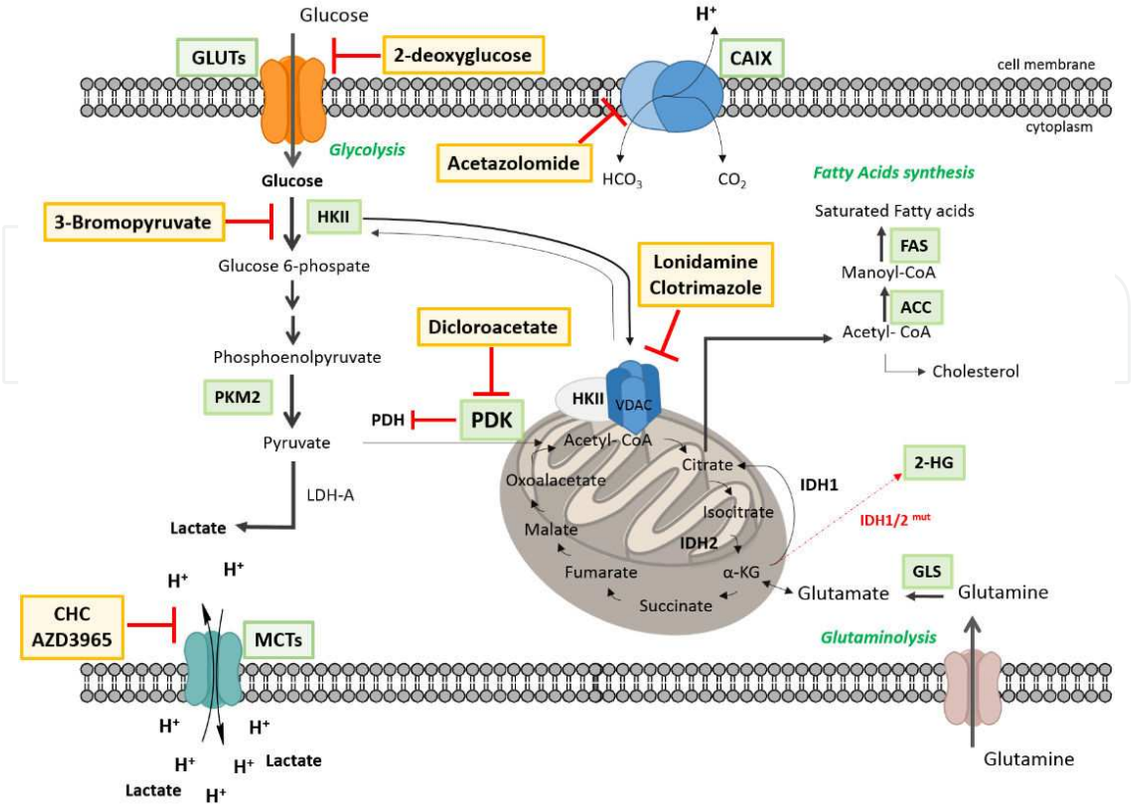

Figure 2. Potential molecular targets in metabolic remodeling of glioblastomas. The green boxes represent the potential metabolic molecular targets in glioblastomas: enzymes involved in glycolytic metabolism, glutamine metabolism, lipid metabolism; different transporters and also the oncometabolite 2-hydroxyglutarate. Yellow boxes represent the different inhibitors of the specific molecular targets described. Abbreviations: GLUTs, glucose transporters; MCTs, monocarboxylate transporters; CAIX, carbonic anhydrase IX; HKII, hexokinase II, PKM2, pyruvate kinase M2; LDH-A, lactate dehydrogenase A; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; IDH1/2, isocitrate dehydrogenase 1/2; IDH1/2 ^{mut}, isocitrate dehydrogenase ½ mutation; 2-HG, 2-hydroxyglutarate; GLS, glutaminase; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; α-KG, α-ketoglutarate; VDAC, voltage-dependent anion channel; CHC, α-cyanohydroxycinnamic acid.

Gonçalves V. *et al*, submitted). Several *in vitro* studies also reported overexpression of GLUT1 expression in GBMs cells when compared with normal astrocytes [32].

These findings raise the importance of GLUT inhibition in tumor therapy, however, at the moment, a glucose transporter inhibitor is not available at the clinical level. Nevertheless, *in vitro* studies have been using 2-deoxy-D-glucose has an inhibitor of glucose uptake promoting a decrease on tumor cell proliferation [33] (Figure 2). These studies reported the high dependence of brain tumors on glucose as source of energy and also for catabolic processes.

2.1.2. Hexokinase II

HK is one of the most important enzymes of the glycolytic pathway, which is responsible for the phosphorylation of glucose to glucose-6-phosphate (G6P), thereby preventing the efflux of glucose from the cell [34]. This enzyme has four isoforms (I-IV) identified in different mammalian tissues [35].

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In most solid tumors, hexokinases type I and II are the most frequently upregulated [36]. In glioblastomas, HKII is highly expressed, whereas HKI is predominantly expressed in normal brain and low grade gliomas [37]. Additionally, HKII is expressed at low levels in neuronal tissue, but is highly expressed in mesenchymal subtype of glioblastomas [37]. As the first enzyme involved in the glycolytic pathway, HK controls glucose flux in glycolysis or the pentose phosphate pathway (PPP) [38]. HKII is a highly regulated form of hexokinase, being regulated by HIF-1α, glucose, p53, insulin, glucagon, cAMP, among others [36]. The four hexokinase types are normally expressed in the cytoplasm, however type I and II can bind to the outer membrane of the mitochondria *via* voltage-dependent anion channel (VDAC) (Figure 2) [39]. The translocation of HKII to mitochondria is regulated by growth factors and signaling pathways, such as EGFR and PI3K/AKT activation, which are known to be upregulated in glioblastomas [40]. Moreover, the association of HKII with mitochondria in gliomas, besides maintaining high glucose influx, also renders cells resistant to apoptosis, due to the prevention of cytochrome c release [41].

Several studies have described that the expression of HKII in gliomas promotes proliferation and increase in lactate production, being dependent on both mitochondrial localization and kinase activity [42]. Additionally, HKII overexpression in glioblastomas confers resistance to treatment with both temozolomide and radiation, being associated with poor overall survival [43]. Furthermore, silencing of HKII in glioma cells leads to decrease in glycolytic metabolism, observed by a decrease in lactate production and increase expression of OXPHOS proteins and oxygen consumption [43]. Finally, it was also demonstrated that reduction of HKII expression impaired tumor growth *in vivo* both on subcutaneous and intracranial xenograft models [37, 43].

Some drugs have been proposed for chemical inhibition of HKII (Figure 2). 3-bromopyruvate (3-BrPA), a pyruvate analogue, is an alkylating agent and also an inhibitor of glycolysis that decreases tumor growth, without apparent toxicity in subcutaneous hepatocellular carcinoma [44]. However, it is effective only at high concentrations (mM) and to the best our knowledge is not under clinical trials. Other known inhibitor is lonidamine, an inhibitor of HKII binding to the mitochondria, which is currently in clinical trials. *In vitro* studies showed a decrease in lactate production in high grade gliomas but not in low grade [33]. Despite that lonidamine treatment leads to a decrease in tumor growth in different solid tumors without adverse effects, the results of a phase I/II efficacy trial was disappointing in gliomas [45, 46]. Clotrimazole is another inhibitor of HKII localization that demonstrated promising results *in vivo*. In gliomas, clotrimazole increased the sensitivity to radiotherapy and also leads to decrease in tumor growth [47, 48].

2.1.3. Pyruvate Kinase (PK)

PK is an enzyme involved in the last irreversible step of the glycolytic pathway, converting phosphoenolpyruvate (PEP) to pyruvate [49, 50]. It is also regulated allosterically by the phosphotyrosine binding or phosphorylation and its expression is regulated by isoform selection [50]. Thus, PKM1 is mostly present in adult tissues, such as adult brain and muscle, whereas PKM2 is more frequent in proliferating tissues and embryonic tissues, namely in fetal

brain and tumor cancer cells [49]. PKM1 and PKM2 presented different properties, which results in different activities. PKM1 is constitutively active, but PKM2 is regulated by fructose-1,6-biphosphate, presenting reduced activity, which allows the accumulation of glycolytic intermediates and promotes the entry of G6P into the oxidative metabolism of PPP for the production of energy and biosynthesis of proteins, lipids and nucleotides (macromolecules) [50-53]. In cancer cells, like glioblastomas, there is upregulation of PKM2 that favors aerobic glycolysis, increasing lactate production [51, 54, 55]. On the other hand, PKM2 favors the biosynthetic pathway, leading to increased biomass. This dual function potentiates tumor proliferation and aggressiveness. The dimeric form of PKM2 delays pyruvate formation and allows the accumulation of upstream glycolytic intermediates for biosynthetic pathways, whereas the tetrameric form favors aerobic glycolysis, increasing lactate production [56].

In lung cancer cell lines, replacing PKM2 by PKM1 decreases lactate production and increases oxygen consumption (reverse Warburg effect) and also decreases the proliferative capacity of cancer cells in nude mice [54]. It was demonstrated in glioblastomas that knockdown of PKM2 decreased cell proliferation and survival but this did not favor the switch from aerobic glycolysis to oxidative phosphorylation, unlike HKII knockdown [43]. Interestingly, PKM2 was identified as essential for survival of glioma stem cells [57].

Another important function of PKM2 has been associated to epigenetic regulation, being a regulator of histone phosphorylation and acetylation of EGFR-driven glioblastomas [58, 59]. Additionally, in glioblastomas, it was demonstrated that PKM2 is involved in the EGFR signaling pathway that induces its phosphorylation and translocation into the nucleus, which in turn promotes activation of the transcription factor *c-Myc* with consequent activation of downstream targets, namely genes involved in glycolytic metabolism [60]. Taken together, inhibition of PKM2, in order to deplete the dimer and tetramer formation, can be a new therapeutic strategy, since it can lead to inhibition of glycolysis, decreasing energy production and at the same time blocking the anabolic process in tumor cells (Figure 2).

2.2. Mitochondrial metabolism in brain tumors

In addition to glycolytic dependence, most tumors present abnormalities in the number and function of mitochondria, as the case of glioblastomas [61]. Otto Warburg hypothesized that the increase on glycolytic metabolism in cancer was due to mitochondrial dysfunction, however nowadays we know that most tumors maintain functional mitochondria [22, 62-64]. Moreover, increased glycolytic metabolism can be a consequence of mitochondrial metabolism impairment, due to abnormalities in components of the tricarboxylic acid (TCA) cycle, alterations in electron transport chain or deficiencies in oxidative phosphorylation [65, 66]. Concerning the selection theory in cancer cells, the dependence on glycolysis occurs gradually in order to compensate the respiratory failure. In contrast to normal brain cells, in which glycolysis and respiration are tightly coupled, tumor cells are defective in their ability to connect glycolysis and respiration [66].

Two mitochondrial enzymes are important in glioblastomas, such as pyruvate dehydrogenase kinase (PDK) and isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2). The presence of mutations in IDH1 and IDH2 has been recently associated with gliomagenesis.

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2.2.1. Pyruvate Dehydrogenase Kinase (PDK)

Pyruvate dehydrogenase (PDH) is a mitochondrial enzyme that controls the entry of pyruvate into mitochondria, promoting its oxidative decarboxylation into acetyl-CoA [67, 68]. The activity of PDH is inhibited by phosphorylation through PDK, resulting in its accumulation in the cytosol and consequent conversion into lactate [67, 68]. PDK is an important mitochondrial matrix protein comprising four isoforms (PDK1 to PDK4), being PDK2 highly expressed in glioblastomas compared to normal adjacent brain tissue [69].

Tumor cells present high levels of glycolysis as a consequence of increased hypoxic microenvironment, which leads to activation of HIF-1α and consequent upregulation of downstream target genes involved in glycolytic metabolism, such as PDK [67]. This enzyme is responsible for the uncoupling between glycolysis and mitochondrial oxidation of glucose, preventing the entry of pyruvate into the mitochondria with consequent increase in glycolytic rates, which confers resistance to apoptosis [67, 68]. Thus, PDK became an important target for glycolytic tumors (Figure 2). Dichloroacetate (DCA), a chemical PDK inhibitor, has been studied in several *in vitro* and *in vivo* models [68]. DCA promotes dephosphorylation of PDH, leading to entry of pyruvate into the mitochondria, decreasing glycolytic rates and lactate production [68, 70]. This leads to activation of oxidative phosphorylation, depolarization of mitochondria and consequent increase in production of reactive oxygen species (ROS), which promotes apoptosis of cancer cells, decreasing tumor cell proliferation [68, 70]. DCA treatment has demonstrated promising results in some tumors, particularly in non-small cell lung, breast and endometrial cancer, either experimentally using *in vitro* and *in vivo* models, as well as in clinical trials [70-73]. In glioblastomas, inhibition of PDK with DCA was evaluated pre-clinically. In C6 glioma cells, it was observed a decrease in lactate production, increase in ROS production, as well as depolarization of mitochondria, which results in a decrease on cell proliferation and induction of apoptosis [74]. *In vivo* it was verified that DCA decreased not only tumor growth but also the angiogenic capacity of glioma cells [74]. The effect of DCA was also tested in a series of glioblastoma patients with congenital acidosis, with a reduction in lactate levels, decrease in HKII localization in the mitochondria, as well as a decrease in mitochondrial polarization, which rendered tumor cells more sensitive to apoptosis [69]. Despite these encouraging results, no exact conclusions can yet be made regarding the efficacy and toxicity of DCA in glioblastoma patients. Thus, a large and randomized clinical study would be important to define the efficacy and toxicity of DCA. Additionally, whether DCA can sensitize GBM cells to temozolomide and radiotherapy remains undetermined.

2.2.2. Isocitrate Dehydrogenase (IDH)

IDH is an enzyme that catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate, generating NADH in the mitochondria or NADPH in the cytoplasm [75]. It is composed by 5 genes, being the *IDH1* and *IDH2* the most explored and important in gliomas. IDH1 is presented in the cytoplasm and peroxisome, whereas IDH2 is presented in the mitochondria [76].

In 2008, recurrent somatic hotspot mutations of *IDH1* and *IDH2* were found in low grade gliomas and secondary glioblastomas [77, 78]. These mutations cause amino acid single change in one of the two alleles of the gene (arginine 132 for *IDH1* and arginine 172 for *IDH2*), being

classified has a dominant mutation [79]. It is described that the arginine mutation occurs in the binding site of the substrate isocitrate [42]. *IDH1* mutations are reported in more than 80% secondary glioblastomas, but only 5% in primary glioblastomas [76, 80, 81]. Additionally, it occurs in 80% of diffuse astrocytomas (WHO grade II). These mutations are more frequent in younger patient secondary glioblastomas, associated with a proneural subtype and also with increased survival [82]. It was reported that the presence of IDH mutations leads to a decrease in α-ketoglutarate that is required for prolyl hydroxylase (PHD) activity that promote degradation of HIF-1α (ref). Thus, downregulation of intracellular α-ketoglutarate contributes to stabilization of HIF-1α, leading to pseudohypoxia [83]. Nevertheless, subsequent studies did not verify an alteration in α-ketoglutarate on mutant IDH1/2, instead, a gain of function activity it was found, which converts α-ketoglutarate to 2-hydroxyglutarate (Figure 2) [84, 85]. The latter has been recognized as an oncometabolite, which inhibits enzymes involved in the α-ketoglutarate pathway. Additionally, it was described that 2-hydroxyglutarate is involved in epigenetic regulation, promoting a hypermethylator phenotype in gliomas [86] and also keep cells in an undifferentiated *status*, or stem cell-like, which can be more permissive to transformation [17]. The presence of pseudohypoxia, due to the constitutive stabilization of HIF-1α, indicates that *IDH1/2* mutations are involved in HIF-1α signaling pathway, which promotes glucose metabolism, angiogenesis and invasion [83]. These results suggest the paramount role of IDH mutations on the metabolic remodeling of glioblastomas, contributing to the "Warburg effect". Therefore, study the involvement of *IDH1/2* mutations in metabolic remodelling and in aerobic glycolysis opens a new window for investigation. Overall, IDH1 is an attractive target for therapy (Figure 2) since they are early events in the progression from low grade to high grade gliomas.

2.3. Glutamine metabolism and lipid synthesis in brain tumors

Like glucose, glutamine is a source of energy for tumor cells, functioning as a nitrogen donor [87, 88]. Glutamine metabolism has been reported to be upregulated in some tumors, being crucial for the biosynthetic processes, namely synthesis of cholesterol and fatty acids [14, 89, 90]. The shift to glutamine metabolism to produce the precursor acetyl-CoA for lipid biosynthesis is a mechanism of adaptation to glycolytic metabolism that prevents the entry of pyruvate into mitochondria, due to upregulation of PDK [91]. In fact, it has been observed an increased expression of glutaminase (GLS) enzyme in tumor cells. GLS is located in the mitochondria and catalyzes the conversion of glutamine to glutamate being transcriptionally regulated by the oncogenes *c-Myc* and *NFκB*. [92-94], An increased concentration of glutamine in glioblastomas compared to normal brain tissue was demonstrated by nuclear magnetic resonance (NMR) [95]. Additionally, there is a low expression of glutamine synthase that correlates with a better prognosis in glioblastomas [96].

Beyond the altered glycolytic and glutamine metabolism in tumor cells, the alteration in lipid metabolism is also recognized as a component of the metabolic reprogramming. It has been observed that tumor cells present reactivation of *de novo* fatty acid synthesis, important for the biogenesis of cellular membranes [97, 98]. Glioblastomas contain higher levels of unsaturated fatty acids compared to normal brain, indicating the presence of exacerbat-

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ed lipogenesis, which is regulated by several key genes, such as SREBP-1 and its downstream-targeted genes acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and low-density lipoprotein receptor (LDLR), which are upregulated in these tumors [99]. Importantly, the EGFR/PI3K/AkT signaling pathway regulates the metabolic reprogramming in glioblastomas [100]. Cholesterol is also an important component of cell membranes and cholesterol esters have been found to be abundantly present in high grade gliomas, but undetectable in normal tissues by NMR techniques [101, 102]. Recently, low density lipoprotein receptor (LDLR) has been described to be upregulated in GBM patients, xenografts and cell lines, and this upregulation was correlated with high levels of cholesterol esters in GBM cells [103]. Interestingly, LDLR is also be upregulated by EGFR/PI3K/Akt signaling, which was been shown to be mediated by SREBP-1 in GBMs [100]. However, little is known about the altered lipid metabolism in cancer cells, namely glioblastomas, and their role in the tumor context, being possible that lipogenesis in cancer cells could support the cell growth located within nutrient-limited areas, thereby contributing to symbiotic relationships within tumors. Once more, lipid metabolism, as well as glutamine metabolism, and their key enzymes are interesting targets in glioblastomas (Figure 2)

3. Lactate transport and pH regulation in brain tumors

A constitutive increase in the glycolytic phenotype of cancer cells leads to acute and chronic acidification of tumor microenvironment. Important proteins involved in acidification of the extracellular space are monocarboxylate transporters (MCTs) that co-transport H+and lactate, and carbonic anhydrases (CAIXs), which are activated by growth factors, oncogenic transformation, hypoxia, and low intracellular pH [21]. As it is known, tumor acidity is associated with cancer cell invasion behavior, i.e. increased migration, invasion and metastasis [104]. Further, tumor acidosis and lactate contributes to several features of tumor progression and malignancy, like immune escape, angiogenesis, and radioresistance, making lactate a key player in cancer aggressiveness. [105]. Still in line with a potential involvement of lactate in the invasion behavior, it has been shown that lactate up-regulates the expression of transforming growth factor (TGF-β2), which is associated with increased migration in glioblastomas [106].

3.1. Monocarboxylate transporters

The MCT family comprises 14 members with similar topology; however, only 4 isoforms (MCT1–MCT4) are proton-linked monocarboxylate transporters, performing the transmembrane transport of monocarboxylates, such as lactate, coupled with a proton, in an equimolar manner [107, 108].

In the last years, several studies reported up-regulation of MCTs in different human solid tumors, showing the importance of MCTs in cancer biology [109]. In brain tumors, the scare studies point to the importance of MCT expression, especially MCT1. Strong expression of MCT1 in the plasma membrane was found in high grade gliomas compared with low-grade

lesions and normal adjacent tissues, which exhibited negative or weak MCT1 staining [110, 111],. A study in neuroblastomas showed, by mRNA quantification, that MCT1 was differently expressed and that its activity was highly associated with MYCN amplification, leading to the hypothesis that expression of MCT1 could be associated with higher malignancy [112]. Further, expression analysis revealed that SLC16A1 transcript, encoding MCT1, was elevated in 90% of the medulloblastomas analyzed [113]. It was also reported that inhibition of MCT activity, particularly MCT1, decreased the glycolytic phenotype (low glucose consumption and lactate production), cell proliferation and invasion, promoting increase in cell death [111, 114, 115]. This elucidates the importance of MCT1 activity in intracellular pH homeostasis and tumor aggressiveness of glioblastomas.

Although MCTs are not the major H^+ transporters, the data available in the literature support the hypothesis of a major contribution of MCTs to the hyper-glycolytic and acid-resistant phenotype, as major adaptation to the hypoxic microenvironment [116]. Thus, MCT inhibition may be a useful therapeutic approach in brain tumors (Figure 2). Actually, it was demonstrated that *in vitro* MCT1 inhibition decreases intracellular pH, leads to cell death and, importantly, enhances cancer cell radiosensivity in gliomas [114, 115]. Importantly, promising results using *in vivo* models have also been reported, where treatment with the chemical inhibitor CHC retarded tumor growth, rendered tumor cells sensitive to radiation and decreased invasion [114, 117]. However, CHC is not a specific MCT inhibitor, having also other targets. Recently, novel MCT1 inhibitors have been designed and may constitute an effective strategy to block MCT1 activity in cancer [118]. A orally administered related compound, AZD3965 (AstraZeneca), is currently in Phase I/II clinical trials for advanced solid tumors [119].

3.2. Carbonic anhydrases

Carbonic anhydrase catalyzes the conversion of extracellular bicarbonate to CO_2 and protons (H^+), thereby contributing to extracellular acidification [120]. This family is composed by 15 isoforms described in mammals, which differ in cellular localization, catalytic activity and susceptibility to different class of inhibitors. Two carbonic anhydrases are overexpressed in many solid tumors, namely CAIX and CAXII, being associated with tumor progression and response to therapy [121]. It is verified that CAIX is mostly negative in normal tissues but increase in the corresponding tumor tissues, whereas CAXII present a diffuse distribution in healthy tissues [122, 123]. Glioblastomas present high levels of intratumoral hypoxia, with consequent HIF-1α activation which contributes to increased expression of glycolysis-related genes [124], including CAIX [125]. CAIX is overexpressed in these tumors with focal plasma membrane expression close to peri-necrotic regions (hypoxic) [126], being negative in normal adjacent tissues, making it a feasible treatment target [127]. Furthermore, it has been described that CAIX is associated to poor overall survival, because it confers resistance to chemotherapy, radiotherapy and anti-angiogenic therapy [128]. Increased expression of CAIX in advanced stages/grades of many tumor types also suggests its association with dedifferentiation [129].

In vitro and *in vivo* approaches have demonstrated the potential of CAIX inhibition (Figure 2). Knockdown of CAIX decreased tumor cell ATP levels under hypoxic and glycolytic

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conditions [126]. In addition, the susceptibility of U251 glioblastoma cells to chemotherapy and radiation treatment was strongly enhanced after CAIX downregulation, which is supported by a recent *in vivo* study [126]. Similarly, CAIX inhibition enhanced the effect of anti-angiogenic therapy with the anti-VEGF antibody bevacizumab [130]. Some inhibitors have been developed to inhibit CA activity, particularly CAIX and CAXII. Acetazolomide, enhances the apoptotic response of glioma cells to temozolomide [131] and an *in vivo* study using derivatives of acetazolamide showed retardation of mice carcinoma xenograft growth after 1 month of treatment [131]. Other studies have identified coumarins as CA inhibitors, however they were not tested yet in the cancer context [132, 133]. Furthermore, specific monoclonal antibodies for the mostly expressed isoforms in tumors, namely CAIX, have been developed, *i.e*, the M75 and WX-G250 for colorectal cancer and renal cell carcinoma, respectively [134, 135].

4. Future perspectives and conclusions

Metabolic transformation plays a major role in gliomas development, tumor progression and adaptation to tumor microenvironment. The interplay between tumor angiogenesis, hypoxia, pH regulation and energy metabolism, glycolysis related enzymes and transporters, as well as pH regulator transporters, may provide promising molecular targets for drug development. In addition to glycolysis, glutaminolysis and fatty acid synthesis represent key metabolic events with potentially interesting drug targets. Furthermore, mutations in *IDH1/2*, detected in a genome wide screen on GBMs, point to new specific transforming events in gliomas. These metabolic pathways are tightly linked and also controlled by signaling events often deregulated in gliomas, underlying the flexibility of glioma cells to develop adaptive mechanisms when exposed to oxygen or nutrient deprivation. This highlights the need of targeting several pathways simultaneously and linking the metabolic targets to the genetic makeup of GBM tumors.

Author details

Vera Miranda Gonçalves[1,2], Fátima Baltazar[1,2] and Rui Manuel Reis[1,2,3*]

*Address all correspondence to: ruireis.hcb@gmail.com

1 Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal

2 ICVS/3B's-PT Government Associate Laboratory, Braga/Guimarães, Portugal

3 Molecular Oncology Research Center, Barretos Cancer Hospital, Barretos, São Paulo, Brazil

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