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Review

Metabolic management of brain cancer ☆

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ABSTRACT

Malignant brain tumors are a significant health problem in children and adults. Conventional therapeutic approaches have been largely unsuccessful in providing long-term management. As primarily a metabolic disease, malignant brain cancer can be managed through changes in metabolic environment. In contrast to normal neurons and glia, which readily transition to ketone bodies (β-hydroxybutyrate) for energy under reduced glucose, malignant brain tumors are strongly dependent on glycolysis for energy. The transition from glucose to ketone bodies as a major energy source is an evolutionary conserved adaptation to food deprivation that permits the survival of normal cells during extreme shifts in nutritional environment. Only those cells with a flexible genome and normal mitochondria can effectively transition from one energy state to another. Mutations restrict genomic and metabolic flexibility thus making tumor cells more vulnerable to energy stress than normal cells. We propose an alternative approach to brain cancer management that exploits the metabolic flexibility of normal cells at the expense of the genetically defective and metabolically challenged tumor cells. This approach to brain cancer management is supported from recent studies in mice and humans treated with calorie restriction and the ketogenic diet. Issues of implementation and use protocols are presented for the metabolic management of brain cancer. This article is part of a Special Issue entitled: Bioenergetics of Cancer.

1. Introduction

Malignant brain cancer is a catastrophic disease of morbidity and mortality in adults and is the second leading cause of cancer death in children [1–7]. Despite advances in imaging technologies, the standard therapies for malignant gliomas today are largely similar to those that have been used for over five decades and generally involve maximal surgical resection followed by chemotherapy with or without radiation therapy [7–12]. About 99% of patients with glioblastoma multiforme also receive perioperative corticosteroids (dexamethasone) as part of the therapy [10]. Although dexamethasone will reduce edema and swelling associated with surgery and radiation [13], it will also elevate circulating levels of blood glucose [14,15]. Glucose is a major fuel for most glycolysis-dependent brain tumor cells and elevated glucose is associated with poor prognosis [16–19]. Radiation therapy produces oxidative tissue damage and necrosis [20–24], which will elevate glutamate levels in the microenvironment [25]. Glutamate is cytotoxic and, through the glutamate-glutamine cycle, will be rapidly metabolized to glutamine by the reactive astrocytes that surround the neoplastic tumor cells [25–27]. Glutamine is a major metabolic fuel for both brain tumor cells and tumor-associated macrophages (TAMs) [28–31]. TAMs release pro-inflammatory and pro-angiogenic factors creating a microenvironment that facilitates aggressive growth of tumor cells [32,33]. While standard therapies manage glioma growth over the short term (weeks to months), they provide an abundance of glucose and glutamine needed for rapid tumor growth and invasion. Ready access to energy metabolites will facilitate glioma recurrence and enhance growth rate over the longer term [33–35]. Indeed, the malignant phenotype of brain tumor cells that survive radiotherapy is often greater than that of the cells from the original tumor.

It is our opinion that the brain of patients with malignant gliomas should rarely be irradiated and that radiation therapy for brain cancer management is largely counterproductive to long-term patient survival [34]. This opinion does not mean that radiation therapy has no redeeming value for patients suffering malignant brain cancer. Of course radiation therapy can increase patient survival over the "no therapy" option. Radiation therapy can also be as good or better than chemotherapy alone [36]. Our point is whether radiation therapy would be better than non-toxic metabolic therapy for long-term brain cancer

Abbreviations: DR, dietary restriction; CR, caloric restriction; KD, ketogenic diet; RKD, calorically restricted ketogenic diet

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management. The issue could be settled with clinical trials where patients receive metabolic therapy in the absence of radiation therapy.

Conventional chemotherapy has faired little better than radiation therapy for the long-term management of malignant brain cancer [8,37–39]. Brain tumor chemotherapy is often associated with adverse effects that diminish the length or quality of life [12,37,38,40]. Like radiation therapy, the widely used drug temozolomide can also enhance necrotic damage in brain tissue [24]. This will contribute to the availability of glutamate and glutamine needed for tumor progression. In an initial study, bevacizumab (Avastin) with irinotecan therapy for malignant brain cancer management killed 6% of those taking the drug, while an additional 38% of patients had to discontinue use due to toxicity issues [40]. Despite the severity of these adverse effects, the investigators considered the marginal response to bevacizumab therapy superior to that of other available anti-angiogenic drug therapies. More recent studies indicate that bevacizumab enhances the invasive properties of already highly invasive brain tumors [39,41]. Indeed, bevacizumab is capable of enhancing the invasive properties of the U87-MG human glioma, which is widely recognized as a noninvasive tumor when grown as a xenograft [41]. Will it be better for patients not to take bevacizumab or to develop new drugs that inhibit bevacizumab-induced invasion? Recent studies also suggest that some anti-angiogenic compounds block chemotherapeutic drug delivery [42,43]. Viewed collectively, these findings indicate that most conventional radiation and brain cancer chemotherapies can enhance glioma energy metabolism and invasive properties, which would contribute to tumor recurrence and reduced patient survival [34].

The therapeutic targeting of brain tumor-associated mutations, while conceptually appealing, may also be problematic as hundreds of mutations can be found in tumors and not all neoplastic cells within the tumor express the same mutations [44,45]. Many targeted gene therapies suffer from the misconception that mutations cause cancer and that therapies targeting the effects of individual mutations will be effective in managing tumor growth [38,46]. These misconceptions have lead to the idea that cancer therapy can be personalized by targeting signaling pathways unique to an individual's tumor. While this therapeutic strategy could be effective for those tumors derived from germ line mutations or situations where most neoplastic cells within the tumor express the same genetic defect, most brain tumors do not arise from germ line mutations and genetic heterogeneity is common within most aggressive tumors [46,47]. Most tumor-associated mutations arise as epiphenomena of tumor progression and their association with causality and pathobiology is far from clear [33,44,48–52]. It is therefore unlikely that targeting brain tumor-associated mutations will have major therapeutic effect for most brain cancer patients.

2. Application of metabolic control theory to brain cancer management

We contend that all cancer regardless of tissue or cellular origin is a disease of abnormal energy metabolism [48]. As such, the non-toxic targeting of tumor cell energy metabolism becomes an attractive alternative to the current standard of care for brain cancer management. Principles of metabolic control theory/analysis can provide the general concepts associated with therapeutic strategies that target tumor cell energy metabolism. Basically, metabolic control analysis evaluates the degree of flux in metabolic pathways and can be used to analyze and treat complex diseases [53–60]. The approach is based on findings that compensatory genetic and biochemical pathways regulate the bioenergetic potential of cells and ultimately the phenotype. As rate-controlling enzymatic steps in biochemical pathways are dependent on metabolic environment, the management of disease phenotype depends more on the flux of the entire system than on the flux of any specific metabolic pathway or metabolite. In other words, complex disease phenotypes can be managed through self-organizing networks that display system wide dynamics involving oxidative and non-oxidative (substrate level) phosphorylation [19,48,61–64]. Global manipulations of these metabolic networks can restore orderly adaptive behavior to widely disordered states involving complex gene-environmental interactions like cancer.

As abnormal energy metabolism and biological chaos characterize brain tumors [8,19,33,65–67], general principles of metabolic control analysis can be effective for brain cancer management. This hypothesis is based on differences in energy metabolism between normal brain cells and neoplastic tumor cells. As long as brain tumors are provided a physiological environment conducive for their energy needs they will survive; when this environment is restricted or abruptly changed they will either grow slower, growth arrest, or perish [8,19]. In this review we describe how calorie restricted diet therapies, which lower circulating glucose and elevate ketone bodies (acetoacetate and β-hydroxybutyrate, β-OHB), can target brain tumors while enhancing the metabolic efficiency of normal neurons and glia. New information also suggests that ketones are toxic to some human tumor cells and that ketones and ketogenic diets might restrict availability of glutamine to tumor cells [68–70]. The success of this therapeutic strategy is also based in large part on the principles of evolutionary biology involving adaptability and variability selection. The information presented in this review has been compiled in part from information that we presented previously [8,19,71,72].

3. Adaptability and variability selection

According to Rick Potts of the Smithsonian Institution, the evolutionary success of our species has been due largely to the germ line inheritance of traits that bestowed adaptive versatility [73,74]. These traits were honed over millions of years and enabled humans to adapt rapidly to abrupt changes in the physical environment. The adaptability to abrupt environmental change is a property of the genome, which was selected for in order to ensure survival under environmental extremes. This hypothesis is an extension of Darwin's original theory (Chapter IV, Natural Selection) and can be applied to the individual cells of the organism, which exist as an integrated society of cells [75]. The success in dealing with environmental stress and disease is therefore dependent on the integrated action of all cells in the organism. Further, this integrated action depends on the flexibility of each cell's genome, which responds to both internal and external signals according to the needs of the organism. More specifically, only those cells possessing flexibility in nutrient utilization will be able to survive under nutrient stress. Environmental forcing has therefore selected those genomes most capable of adapting to change in order to maintain metabolic homeostasis [19,73–75].

The widely held notion that tumor cells are more “adaptable” or have a “growth advantage” over normal cells is inconsistent with evolutionary theory [19]. How can tumor cells that express multiple random pathogenic mutations, chromosomal rearrangements, and mitochondrial abnormalities be considered more “fit” or “advantaged” than normal cells that possess a flexible genome, normal respiratory capacity, and adaptive versatility? The answer is they are not. The issue is metabolic flexibility that is inherited through the genome verses perceived metabolic adaptability that is acquired somatically. Metabolic flexibility allows the organism to respond in a coordinated way to environmental stress according to Darwin's original theory. Although germ line changes could give some organisms a selective advantage when confronted with a novel environmental stress, most mutations reduce fitness. The genomic changes in cancer cells are not inherited in the germ line, but are acquired randomly [45,46]. Tumor cells survive in hypoxic environments not because they have inherited genes making them more fit or adaptable than normal cells, but because they have damaged mitochondria and have thus acquired the ability to derive energy largely through substrate level phosphorylation [48]. Energy through substrate level phosphorylation is required for survival in hypoxia [76–78]. Tissue macrophages can also survive in hypoxic (acidic) environments, as a part of their normal function. Do neoplastic cells have a selective advantage over tissue

macrophages, which evolved to function in hypoxic environments? The energy transition from oxidative phosphorylation to substrate level phosphorylation requires the activation of oncogenes and the inactivation of tumor suppressor genes [48]. What appears as a growth advantage is actually an abnormal phenotype of dysregulated cell growth [48]. Ammonia (NH_3) released from glutamine metabolism could also neutralize lactic acid acidity in the microenvironment by forming NH_4^+ [28,30,79]. Normal cells can also grow faster than tumor cells during normal wound repair [76]. If the acquired mutations expressed in tumor cells provided a selective advantage, then tumor cells should adapt to environmental and metabolic stress better than the normal cells, which do not contain these mutations. The greater vulnerability of tumor cells than normal cells to dietary energy restriction argues against the hypothesis that tumor cells are more "adaptable" or "selectively advantaged" over normal cells.

Cancer cells survive and multiply only in physiological environments that provide fuels (mostly glucose and glutamine) subserving their requirement for substrate level phosphorylation [48]. If these fuels become restricted, tumor cells will have difficulty surviving and growing. Multiple genetic defects will reduce genomic flexibility thus increasing the likelihood of cell death under environmental stress. Regardless of when or how genomic defects become involved in the initiation or progression of brain tumors, these defects can be exploited for the destruction or management of the tumor. In other words, the types and kinds of genetic mutations expressed in brain tumor cells are largely irrelevant in our approach to brain tumor management. How can mutations be relevant to the nature of disease if the complement of mutations differs from one neoplastic cell to the next within most tumors of non-germline origin [44–46]? Although common gene mutations occur in some tumors, it is unlikely that these mutations are expressed in every individual cell of the tumor due to the cellular and genetic heterogeneity. The data from Lobe and colleagues make this fact abundantly clear [45]. It is nevertheless interesting that glioma progression is generally slower in patients with chromosome 1p/19q co-deletions, promoter hypermethylation of the O^6-methylguanine methyltransferase (*MGMT*) gene, or mutations in the gene for isocitrate dehydrogenase1 (*IDH1*) [80–82]. Are we to consider these as "good" mutations? Would targeting these genes reduce or enhance patient survival? Considering the complexity of metabolic flux, genetic heterogeneity, and gene-environmental interactions [47,58,59,61,83], caution should be used in thinking that targeting any specific mutation or pathway will have major effect on brain tumor growth or patient survival. Our perspective is based on emerging evidence that cancer is primarily a metabolic disease that can be managed through systemic metabolic therapy [48]. Recent findings using restricted diets, that produce systemic energy stress, provide direct support for our hypothesis [18,62,84–88].

4. Energy metabolism in brain tumors

Major physiological changes occur during therapeutic fasting in humans or dietary energy restriction in mice. Generally, insulin and glucose levels become reduced, while glucagon, free fatty acids, and ketone bodies (β-hydroxybutyrate and acetoacetate) become elevated [89–91]. While glucose is the preferred energy substrate of normal neurons and glia, these cells will metabolize ketone bodies for energy under fasting-induced reductions of blood glucose. Due to limited uptake from the circulation, free fatty acids are not extensively metabolized for energy by brain cells [89,92]. However, recent work from Kashiwaya, Veech and co-workers showed that fatty acids could be metabolized for energy in rat brain [68]. Brain ketone body metabolism is a conserved physiological adaptation to prolonged food restriction and evolved to enhance survival and maintain adequate neural functions while sparing proteins [89,92–97]. In contrast to normal brain, which can oxidize either glucose or ketone bodies for energy, malignant brain tumors from either humans or animal models lack metabolic flexibility and are heavily dependent on glucose for energy [18,30,66,98–105]. Enhanced glycolysis produces excess lactic acid that can return to the tumor as glucose through the Cori cycle [106]. Although some neural tumors metabolize ketone bodies, this metabolism could be more for lipid synthesis than for energy production [107,108]. Many neural tumors also have reduced activity of succinyl-CoA: 3-ketoacid CoA transferase, the rate-controlling step for utilizing β-OHB as a respiratory fuel [62,70,109–111]. Defects in this enzyme will limit the ability of tumor cells to utilize ketone bodies as an alternative fuel to glucose. Hence, metabolic stress following the gradual replacement of glucose with ketone bodies will be greater in tumor cells than in normal cells.

In addition to glucose, glutamine can also provide energy to tumors through replenishment of TCA cycle metabolites (anaplerosis) [30,112,113]. Glutamine, after metabolism to α-ketoglutarate, can also provide energy through substrate level phosphorylation within the TCA cycle itself [77,78,114]. TCA cycle substrate level phosphorylation could, together with glycolysis, provide sufficient energy for tumor cells with defective oxidative phosphorylation [48,115]. Ketones could also reduce the activity of succinyl-CoA synthetase (SCS), which is required for TCA cycle substrate level phosphorylation under hypoxia [78]. Ketones could therefore indirectly target ATP production from glutamine metabolism. Considered together, these studies indicate that brain tumors, like most malignant tumors, depend heavily on substrate level phosphorylation for their metabolic energy and either lack or have reduced capacity to metabolize β-OHB for energy.

5. Mitochondrial defects in brain tumors

Besides glycolytic dependence, most tumors including brain tumors, express abnormalities in the number and function of their mitochondria [19,48,101,116–123]. Such abnormalities would prevent the utilization of ketone bodies for energy production since functional mitochondria are necessary for ketone oxidation [62,124]. Integrity of the inner mitochondrial membrane is necessary for ketone body metabolism since β-hydroxybutyrate dehydrogenase, which catalyzes the first step in the metabolism of β-OHB to acetoacetate, interacts with cardiolipin and other phospholipids in the inner membrane [116,125,126]. Inner membrane breakdown (cristolysis) and cardiolipin abnormalities characterize many tumors including gliomas [48,116,122,127]. These findings further suggest that glioma cells will be unable to generate adequate levels of energy for survival if ketone bodies become a major energy fuel for the brain.

Otto Warburg originally emphasized that the high glycolytic rate of tumors resulted from diminished or disturbed respiration [76,128]. While most cells die from damaged respiration, those cells that can enhance and modify their anaerobic glycolysis in response to respiratory damage will survive and form tumors. Later studies in a variety of neural and non-neural tumor systems showed that these respiratory disturbances involve abnormalities in TCA cycle components, alterations in electron transport, and deficiencies in oxidative phosphorylation [48,105,113,121,129–132]. While mitochondrial DNA mutations can also diminish respiration, many described tumor mtDNA mutations may be artifacts of interpretation or appear to be non-pathogenic [133,134]. We purified and sequenced the entire mitochondrial genome from isolated mitochondria in five independently derived mouse brain tumors, but were unable to find a single pathogenic mutation in any of the tumors [134]. Structural defects of the inner mitochondrial membrane, that would alter the proton motive gradient, would also prevent normal ATP production despite the appearance of oxidative metabolism, i.e., oxygen consumption and CO_2 production [48,116,122,130,135–137]. In other words, the mitochondria of many gliomas and most tumors for that matter are dysfunctional. Although electrons may be transferred, this transfer is not effectively coupled to oxidative energy production. The bulk of experimental evidence

indicates that mitochondria are dysfunctional in tumors and incapable of generating sufficient ATP through oxidative phosphorylation [48,116].

6. Mitochondrial lipid abnormalities in murine brain tumors

Our recent data show that murine gliomas contain numerous defects in the content and composition of mitochondrial lipids especially cardiolipin [121,138]. Cardiolipin is essential for efficient oxidative energy production and mitochondrial function [139–154]. The lipid composition of mitochondrial membranes also influences the activity of β-hydroxybutyrate dehydrogenase, which is needed for ketone body metabolism [62,126,155,156]. Any genetic or environmental alteration in the content or composition of cardiolipin will compromise energy production through oxidative phosphorylation [116,118,127,144,157–164]. Cardiolipin defects in tumor cells are also associated with reduced activities of several enzymes of the mitochondrial electron transport chain making it unlikely that tumor cells with cardiolipin abnormalities can generate adequate energy through oxidative phosphorylation [116,121,127,138].

Cardiolipin abnormalities can arise from any number of environmental insults linked to the origin of cancer including radiation, hypoxia, inflammation, etc [121]. Cardiolipin abnormalities can also arise from simply growing mammalian cells in culture. It appears that the *in vitro* growth environment alters cardiolipin composition, reduces Complex I activity, and obscures the boundaries of the Crabtree and the Warburg effects [138]. The Crabtree effect involves the inhibition of respiration by high levels of glucose [165–167], whereas the Warburg effect involves elevated glycolysis from impaired oxidative phosphorylation [76]. While the Crabtree effect can be reversible, the Warburg effect is largely irreversible because its origin is with permanently damaged mitochondria. We found that growth environment produced different types of cardiolipin abnormalities. The tumor-specific abnormalities in cardiolipin composition observed in the *in vivo* environment were largely different from the abnormalities found in the tumor cells when grown in culture [138]. Moreover the cardiolipin composition of cultured brain tumor cells was similar to that of syngeneic non-tumorigenic astrocytes indicating that the *in vitro* environment produces cardiolipin abnormalities independent of those associated with tumorigenesis. This was also supported by findings that lactic acid production was similar in non-tumorigenic astrocytes and glioma cells [138]. Since energy production through oxidative phosphorylation is dependent on cardiolipin composition, abnormalities in cardiolipin composition whether genetic or non-genetic will inhibit respiratory energy production.

We consider it unlikely that the cardiolipin and mitochondrial lipid abnormalities of gliomas can be corrected so that the cells could respire normally [168]. The irreversible nature of the problem resides in the complex process of cardiolipin remodeling required for normal oxidative phosphorylation [116,169]. Moreover, we think that the numerous somatic genetic mutations in tumor cells will "lock in" the mitochondrial lipid abnormalities making complete restoration of normal energy metabolism and the differentiated state highly unlikely. Our findings from multiple murine brain tumors support the Warburg theory of cancer as a disease of mitochondrial energy metabolism [48,121].

The continued production of lactic acid in the presence of oxygen is the metabolic hallmark of most cancers and is referred to as aerobic glycolysis or the Warburg effect. Interestingly, Warburg considered aerobic glycolysis as an epiphenomenon of tumor progression that was too labile or too dependent on environmental conditions to be reliable indicator of tumor metabolism [19,76]. This came from findings that oxygen consumption and CO_2 production in tumor cells was not associated with coupled respiration. Rather, Warburg emphasized the importance of defects in the coordination of glycolysis with respiration. The latency between tumor initiation and progression was considered the period necessary for glycolysis to compensate for the impaired respiratory function. Warburg clearly showed that glycolysis was necessary to maintain cell viability when respiration was impaired [76,128]. Considerable effort is now underway to explain the genetic and biochemical basis of the Warburg effect [48,117,170–184]. If respiration is damaged in tumor cells, it should be no surprise that expression of many genes associated with glycolytic pathways will be upregulated [48,85,117]. This upregulation is necessary for tumor cell survival. As Warburg clearly showed, aerobic glycolysis becomes a necessary compensatory energy source following respiratory damage [76]. We recently described how the retrograde signaling system could induce changes in oncogenes and tumor suppressor genes to facilitate tumor cell survival following mitochondrial damage [48].

In addition to glycolysis, glutamine can also increase ATP production under hypoxic conditions through substrate level phosphorylation in the TCA cycle after its metabolism to α-ketoglutarate [77,78,185]. Increased c-MYC expression is associated with increased glutamine metabolism [186,187]. Enhanced energy production through TCA cycle substrate level phosphorylation using glutamine as substrate could give the impression of functional oxidative phosphorylation since ATP is generated within the mitochondria. It is difficult to determine with certainty, however, if mitochondrial ATP production is generated through oxidative phosphorylation or through increased succinyl-CoA synthetase activity in the TCA cycle itself [78]. The later possibility can be considered "pseudo respiration" especially if oxygen is consumed and electron transport is uncoupled. Few investigators make the distinction between the different forms of mitochondrial energy production. TCA cycle substrate level phosphorylation could therefore become another source of ATP production in tumor cells with impairments in oxidative phosphorylation [48]. Our preliminary findings in metastatic tumor cells support this possibility [115].

Our recent findings in a series of spontaneous murine brain tumors suggest that mitochondrial lipid abnormalities, which alter electron transport activities, can account in large part for the Warburg effect [138,168]. These lipid abnormalities would compromise the proton motive gradient thus uncoupling mitochondria. A dependence on glucose and glutamine for survival together with multiple types of mutations and mitochondrial defects makes most tumors potentially manageable according principles evolutionary biology and metabolic control analysis as recently described [19,48,50,188]. Our recent studies with calorie restriction and the restricted ketogenic diet exploit the Warburg effect for the metabolic management of malignant brain tumors [62,84,85,88,189]. We also showed that targeting glutamine could significantly reduce the systemic metastatic spread of brain tumor cells [188]. Although extracranial metastasis is considered rare for malignant brain tumors, there is a large literature indicating that brain tumors are highly metastatic when growing outside the nervous system [190–194]. Some tumors appear to be more dependent on glutamine than glucose for survival and growth, whereas the opposite is the case for other tumors. The difference depends in part on the type of cell from which the tumor arises, with cells of myeloid origin being especially dependent on glutamine. We proposed that targeting both glucose and glutamine metabolism could be effective for managing most cancers including brain cancer [48,188]. The ketogenic diet and dietary energy restriction involving calorie restriction can help to reduce levels of these energy metabolites. When combined with drugs that also target glutamine metabolism, the restricted ketogenic diet can produce a global metabolic management of tumor growth.

7. The ketogenic diet

In 1995, Nebeling and coworkers attempted the first nutritional metabolic therapy for human malignant brain cancer using the ketogenic diet [195]. The ketogenic diet (KD) is a high fat low carbohydrate diet that has been used for decades as an effective therapy for refractory seizures in children [19,196–198]. The objective of the study was to shift the prime substrate for energy metabolism from glucose to

ketone bodies in order to disrupt tumor metabolism while maintaining the nutritional status of patients [195]. The patients in this landmark clinical study included two female children with nonresectable advanced stage brain tumors (anaplastic astrocytoma stage IV, and cerebellar astrocytoma stage III). Measurable tumor remained in both subjects following extensive radiation and chemotherapy. Although severe life threatening adverse effects occurred from the radiation and chemotherapy, both children responded remarkably well to the KD and experienced long-term tumor management without further chemo or radiation therapy. Indeed, one of the patients remains alive at the time of this writing (Nebeling, personal communication). Positron Emission Tomography with fluro-deoxy-glucose (FDG-PET) also showed a 21.8% reduction in glucose uptake at the tumor site in both subjects on the KD [195]. These findings indicate that a restricted ketogenic diet, which lowers glucose and elevates ketone bodies, could reduce glycolytic energy metabolism in these brain tumors. More recently we published a case report showing that a modified ketogenic diet could help manage glioblastoma growth in an older female patient [84]. These findings show that the ketogenic diet, when consumed in restricted amounts, is well tolerated and can be an effective non-toxic therapy for malignant brain cancer in both children and adults.

Despite the efficacy of this metabolic approach to brain cancer management, no clinical trials have been initiated in the United States to date on the therapeutic efficacy of restricted ketogenic diets (RKD) for managing brain cancer in either children or adults. What is the reason for not initiating clinical trials on the RKD for brain cancer management? Some have suggested that the North America Brain Tumor Collaborative prefers "hand-me-down" drug therapies from other cancer studies rather than exploring less costly and more effective alternative approaches [12,19]. This is unfortunate for patients, as our recent findings in brain tumor animal models show that the therapeutic potential of the RKD, involving reduced glucose and elevated β-OHB, could be superior to that of most current brain tumor therapies [62,72,88]. Moreover, the RKD would eliminate or greatly reduce the need for adjuvant anticonvulsant and steroidal medications (dexamethasone) for brain tumor patients. The RKD was designed initially as an antiepileptic therapy and can therefore be used to manage tumor-associated seizure activity [196,198–202]. It is unclear why brain cancer patients are given anticonvulsant medications when the KD can achieve the same clinical endpoint while also targeting tumor growth. A clinical trial using the KD for recurrent glioblastoma has been initiated in Germany (ERGO trial) under the direction of J. Rieger at the University of Tübingen. Modest improvement was reported without adverse effects, but no published information was presented on blood glucose or blood ketone levels in the treated patients. This information is needed to gage the degree of energy stress on surviving tumor cells. Unfortunately, most patients in this trial suffered through the conventional standard of care before receiving the KD. We consider the current standard of care (primarily involving radiotherapy and dexamethasone) as counter productive to long-term patient survival [34]. Preliminary findings in humans and mice indicate that the RKD could be an effective nontoxic therapy for malignant brain cancer [19,62,84,195].

8. Dietary energy restriction

We confirmed the findings of the Nebeling group in a series of orthotopic mouse brain tumor models treated with the RKD and dietary energy restriction [18,62,86–88] (Fig. 1). As with the KD, dietary restriction (DR) reduces glucose and elevates ketone bodies [18,63,64,91]. The DR-induced inhibition of brain tumor growth is directly correlated with reduced levels of glucose and elevated levels of ketone bodies [18]. The gradual transition from glucose to ketone bodies as an energy source is key for the longer-term management of brain tumors. The transition requires multiple gene and metabolic adjustments, which tumor cells lack due to their accumulated mutations. DR is produced from a total restriction of dietary nutrients and differs from starvation in that DR reduces total calorie energy intake without causing anorexia or malnutrition [55,203–207]. As a natural therapy, DR improves health, prevents tumor formation, and reduces inflammation [55,205,208–211]. Hence, reduced calorie intake is ideally suited as a therapy for managing brain cancer without adverse effects.

Previous studies showed that the anti-tumor effects of DR result more from calorie restriction *per se* then from the restriction of any specific dietary component such as proteins, vitamins, minerals, fats, or carbohydrates [18,206,207,212]. Reduced dietary copper levels, however, could reduce angiogenesis [213]. Caloric restriction, which lowers glucose and elevates ketone bodies [63,64], improves mitochondrial respiratory function and glutathione redox state in normal cells [56,214,215]. Ketone bodies can also protect normal neurons and glia from damage associated with aggressive tumor growth through a

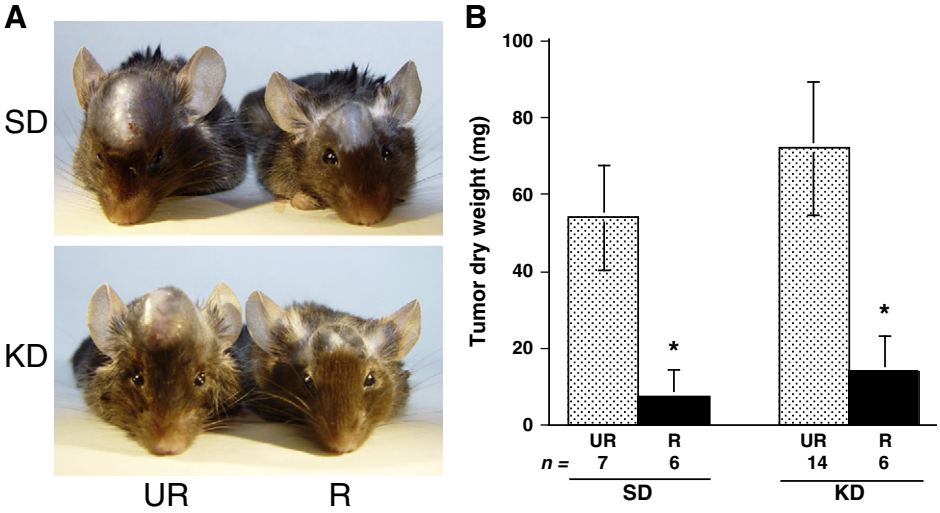

Fig. 1. Influence of diet on the intracerebral growth of the CT-2A brain tumor. The visual representation (A) and quantitative assessment (B) of the tumor growth in C57BL/6J mice receiving the standard high carbohydrate diet (SD) or the ketogenic diet (KD) under either unrestricted (UR) or restricted (R) feeding as we described [86,88]. Values in B are expressed as means with 95% confidence intervals, and n = the number of mice examined in each group. The dry weights of the tumors in R group were significantly lower than those in the UR group at $P<0.01$. The results show that DR significantly reduces tumor growth. No adverse effects were seen in the mice maintained on the 30%–40% DR. Despite a reduction in total body weight, the DR-fed mice were more healthy and active than the AL-fed mice as assessed by ambulatory and grooming behavior. No signs of vitamin or mineral deficiency were observed in the DR-fed mice according to standard criteria for mice. These findings are consistent with the well-recognized health benefits of mild to moderate diet restriction in rodents [86,88].

variety of neuroprotective mechanisms including elevated glutathione levels [96,124,216–223]. Although elevated ketone bodies are often associated with diabetic states, ketone body elevation in people with normal physiology is considered "good medicine" and therapeutic for a broad range of cardiac, neurological, and neurodegenerative diseases [57,91,94,96,224]. There is also a recent report indicating that ketone bodies inhibit viability of human neuroblastoma cells, but not of normal cells [70]. These findings indicate that elevation of ketone bodies in individuals with normal physiology can be toxic to tumor cells while therapeutic to normal cells.

DR naturally inhibits glycolysis and tumor growth by lowering circulating glucose levels, while at the same time, enhancing the health and vitality of normal cells and tissues through ketone body metabolism [62,85,224]. It is important to recognize, however, that the physiological response to DR is not the same in mice and humans due to differences in basal metabolic rate. The health benefits documented in mice under 40% DR can be realized in humans under very low calorie intake (400–500 kcal) or with water only therapeutic fasting [91]. Alternatively, these health benefits can also be achieved using the restricted KD, which increases circulating levels of ketone bodies while maintaining low blood glucose levels [62,64]. Recent studies from Kashiwaya, Veech and co-workers suggest that diets supplemented with ketone esters could also be effective in reducing blood glucose and glutamine while elevating ketone levels [68].

9. Dietary restriction is antiangiogenic and proapoptotic

Payton Rous first suggested that DR inhibited tumor growth by delaying tumor vascularity (angiogenesis) from the host [225]. Angiogenesis involves neo-vascularization or the formation of new capillaries from existing blood vessels and is associated with the processes of tissue inflammation, wound healing, and tumorigenesis [226–228]. A significant literature suggests that vascularity is rate limiting for the formation of solid tumors, including brain tumors [227,229–233]. The malignancy and invasiveness of brain tumors is also correlated with the degree of their vascularity since prognosis is generally better for tumors that are less vascular than for those that are more vascular [230,234,235]. Inhibition of vascularity is therefore considered an important therapeutic strategy for managing brain tumors [40,229,236–238]. The challenge is to target tumor angiogenesis without harming patients or reducing the quality of life.

We corroborated the Rous hypothesis in our mouse and human (U87-MG) brain tumor models by showing that DR is anti-angiogenic (Fig. 2 and Table 1). Biomarkers for angiogenesis, to include insulin-like growth factor 1 (IGF-1) and vascular endothelial growth factor (VEGF) were significantly lower in all tumors when grown under DR than when grown under unrestricted or ad libitum conditions [87]. DR also reduces angiogenesis in prostate and breast cancer [212,239]. As DR targets brain tumor angiogenesis naturally, while also enhancing the health and vitality of normal brain cells, we suggest that the anti-angiogenic effects of DR or calorically restricted ketogenic diets will be superior to that of most known anti-angiogenic drug therapies for brain tumors including those involving metronomic applications, where multiple anti-angiogenic drugs are given together [240]. In light of our findings, it is surprising that the field would persist in treating brain cancer patients with toxic anti-angiogenic drugs that show marginal efficacy.

Besides reducing angiogenesis, DR also significantly increases brain tumor apoptosis or programmed cell death [86,87] (Table 1 and Fig. 2). This was associated with enhanced caspase-3 activation and poly(ADP-ribose) polymerase cleavage in mouse brain tumors. The proapoptotic

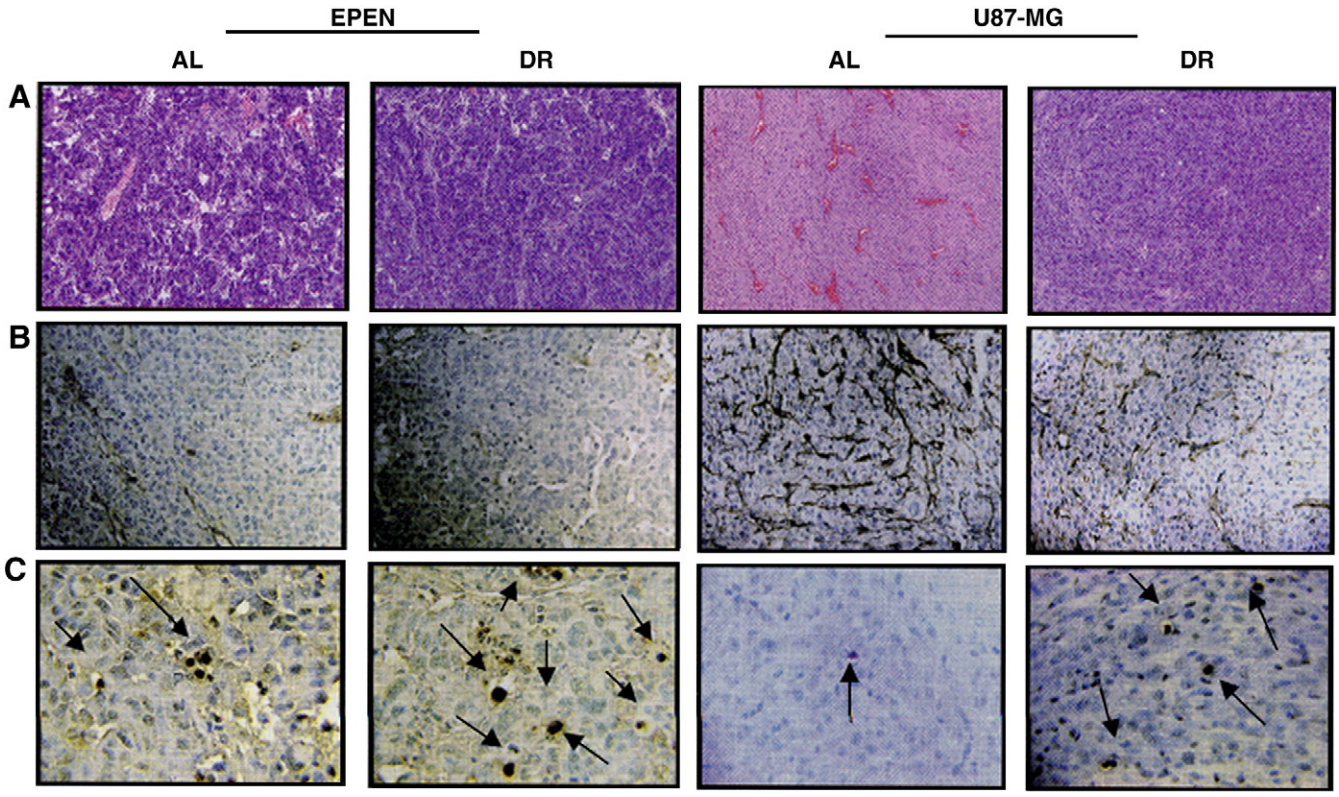

Fig. 2. Influence of dietary restriction (DR) on morphology, microvessel density, and apoptosis in the mouse ependymoblastoma (EPEN) and the human malignant glioma (U87-MG). DR was initiated as we previously described [87]. H&E stained tumor sections in an *ad libitum-fed* (AL) mouse and in a DR mouse (A) (100×). Factor VIII immunostaining from the tumor grown in an AL mouse and in a DR mouse (B) (200×). TUNEL positive apoptotic cells (arrows) from the tumor grown in an AL mouse and in a DR mouse (C) (400×). Each stained section was representative of the entire tumor. All images were produced from digital photography. The findings show that DR is anti-angiogenic and proapoptotic. Reprinted with permission from Clinical Cancer Research [87]. DR had similar effects on the CT-2A malignant astrocytoma as we previously showed [86].

Table 1
Effects of dietary restriction on biomarkers for vascularity and apoptosis in the CT-2A, EPEN and U87-MG brain tumors.

Tumors	Diet[a]	MVD[b]	Apoptosis[c] %	Proliferation[d] %	IGF-1 (ng/ml)	VEGF (pg/ml)
CT-2A	AL	24.3 ± 1.4 (5)[e]	3.7 ± 0.4 (5)	71 ± 3 (5)	273 ± 63 (12)	118 ± 17 (5)
	DR	10.3 ± 3.1** (5)	8.1 ± 1.2** (5)	68 ± 2 (5)	170 ± 29** (17)	80 ± 17* (5)
EPEN	AL	7.7 ± 2.4 (6)	3.4 ± 0.9 (6)	48 ± 3 (3)	149 ± 19 (4)	86 ± 19 (4)
	DR	3.6 ± 1.2* (5)	8.1 ± 2.9** (5)	43 ± 2 (3)	77 ± 44** (4)	94 ± 43 (4)
U87-MG	AL	51.0 ± 9.4 (7)	0.9 ± 0.1 (3)	85 ± 5 (3)	370 ± 134 (5)	136 ± 22 (5)
	DR	28.3 ± 3.3** (3)	3.7 ± 1.8* (3)	65 ± 5** (3)	158 ± 25** (6)	100 ± 8* (7)

[a] Animals were fed either ad libitum (AL) or under dietary restriction (DR) as described in Methods. All values are expressed as means ± 95% CI. The asterisks indicate that the values from the DR group differed from AL group at $P<0.05$ *$P<0.01$ ** as determined by ANOVA. The details for each measurement and statistics are described in [87].

[b] Microvessel density-Factor VIII positive microvessels were averaged in three hotspot areas of each tumor section per high power field.

[c] Apoptotic index % as determined by TUNEL assay.

[d] Proliferation index % as determined by PCNA assay.

[e] Numbers in parentheses represent the number of independent samples analyzed. With permission from Clinical Cancer Research [87].

effects of DR occur in large part from reduced glycolytic energy that most tumors rely upon for growth [85,99,241]. DR can kill tumor cells by depleting available energy or by creating tumor-specific oxidative stress through glucose deprivation [19,242]. In contrast to producing oxidative stress in tumor cells, DR will reduce oxidative stress in normal cells through elevation of ketone bodies [216–219]. The widely held notion that tumor cells are resistant to apoptosis is inconsistent with our findings that DR enhances tumor cell apoptosis. We suggest that apoptosis resistance arises largely from enhanced substrate level phosphorylation of tumor cells and to the genes associated with elevated glycolysis and glutaminolysis, e.g., c-Myc, Hif-1a, etc, which inhibit apoptosis [48,85,243]. If energy from glycolysis and glutaminolysis is reduced, then many tumor cells will die or growth arrest from energy deprivation. DR is a simple natural process by which tumor glycolysis can be targeted without causing toxicity to normal cells. Restricted ketogenic diets can also reduce availability of glutamine to brain tumors since ketone bodies and the KD enhances glutamine export from the brain [69]. Recent studies also show that ketone ester diets, which elevate blood ketones, can also reduce brain glutamate and glutamine, while reducing food intake and blood glucose levels [68]. Hence, DR with elevated ketones is a remarkably simple therapy for targeting either glucose or glutamine metabolism in tumors.

In addition to showing that the CT-2A astrocytoma shares several genetic and biochemical properties with that of human astrocytomas, we found that *late*-onset DR (i.e. DR initiated 10 days after tumor implantation rather than only 2–3 days after) could reduce tumor growth, delay malignant progression, and significantly extend mouse survival [85] (Fig. 3). These findings emphasize an important role for autocrine/paracrine activation of the IGF-I/Akt signaling pathway in potentiating the anti-apoptotic phenotype of astrocytomas and suggest that DR targets this signaling pathway. The DR-induced reduction of glycolysis, evidenced by declines in both circulating glucose and lactate levels as well as in the expression of hypoxia-inducible factor-1α (HIF-1α) and the type 1 glucose transporter (GLUT1), was also associated with a reduction in signaling through the IGF-I/Akt pathway [85]. Reduced glycolytic energy could increase ROS-related cell death in tumor cells while reducing ROS levels in normal cells [242]. Normal cells switch to ketone bodies for energy under low glucose, which reduces ROS production [57,89,216]. The reduction the GLUT1 transporter in the CT-2A astrocytoma cells under DR is opposite to the response of normal brain cells, which increase expression of GLUT1 [85]. If the CT-2A cells were more fit or adaptable than normal cells then GLUT1 expression would be expected to increase more in tumor cells than in normal cells. This was clearly not the case and indicates a differential response to energy stress in normal cells and CT-2A tumor cells. Reduction of IGF-1 expression can be lethal to glycolysis-dependent tumor cells, but not harmful to normal cells [18,85,87,244]. Recent studies show that dietary energy restriction enhances phosphorylation of adenosine monophosphate kinase (AMPK), which induces apoptosis in glycolytic-dependent astrocytoma cells, but protects normal brain cells from death [245]. Viewed together, these findings illustrate further that a shift in energy metabolism from glucose to ketone bodies protects respiratory competent normal cells while targeting the genetically defective and respiratory challenged tumor cells, which depend more heavily on glycolysis than normal cells for survival [48,85].

It is important to mention that tumor growth site and host might influence the therapeutic action of DR against brain cancer. For example, we found that DR significantly reduces the growth of the PTEN-deficient CT-2A malignant mouse astrocytoma and the human U87-MG glioma, which have PI3K activation [85,246]. However, our findings with these tumors differ from the findings in a more recent report showing that DR was ineffective in reducing the growth of the U87-MG and other human tumors when grown in mice with characteristics of diabetes, i.e., non-obese diabetic/SCID mice [246,247]. In contrast to the Kalaany and Sabatini study, we evaluated tumor growth in the orthotopic site (brain) and in mice that did not have characteristics of diabetes [85,87]. It is therefore possible that the tumor implantation site and type of host could influence the effects of DR on tumor growth. As a broad-spectrum inhibitor of multiple signaling pathways related to apoptosis, angiogenesis, and proliferation, DR should have significant anti-tumor effects *in vivo* regardless of the number and types of mutations expressed in the tumor cells.

Phosphorylation and inactivation of BAD and procaspase-9 mediate, in part, the anti-apoptotic actions of Akt activation [248,249]. BAD transmits pro-apoptotic signals generated during glucose/growth factor deprivation. We found that BAD was constitutively phosphorylated in the CT-2A astrocytoma compared with contralateral normal brain, and showed that DR suppressed BAD phosphorylation and increased procaspase-9/-3 cleavage [85]. BAD stimulates apoptosis by forming heterodimers with and inactivating the anti-apoptotic proteins Bcl-2 and Bcl-xL [248,249]. DR is known to reduce Bcl-2 and Bcl-xL expression and to increase the expression of Bax, Apaf-1, caspase-9, and caspase-3 in experimental carcinomas, suggesting that DR could inhibit tumor growth by inducing mitochondrial-dependent apoptosis mediated by the dephosphorylation of BAD [250]. These findings are consistent with evidence that DR is pro-apoptotic in malignant astrocytomas and support evidence that BAD coordinates glucose/IGF-1 homeostasis and the induction of apoptosis [85,87,245,248,249]. Thus, our findings showed that reduced glucose availability and IGF-1 expression play a key role in suppressing Akt and in mediating the pro-apoptotic effects of DR in a PTEN/TSC2-deficient mouse astrocytoma [85] (Fig. 4).

10. Dietary restriction is anti-invasive in experimental glioblastoma

It is the highly invasive nature of malignant brain tumors that makes them difficult to manage using most conventional therapies. Although restricted ketogenic diets can be effective in managing invasive brain cancer in children and adults [84,195], few studies have evaluated the therapeutic effect of calorie or dietary restriction on invasive brain cancer in mice. The invasive properties of many malignant human brain tumors follow the "secondary structures of Scherer," which include diffuse parenchymal invasion, perivascular growth, subpial surface growth, and growth along white matter tracts [251,252]. We recently showed that the VM-M3 invasive glioblastoma model, which was derived from a spontaneous brain

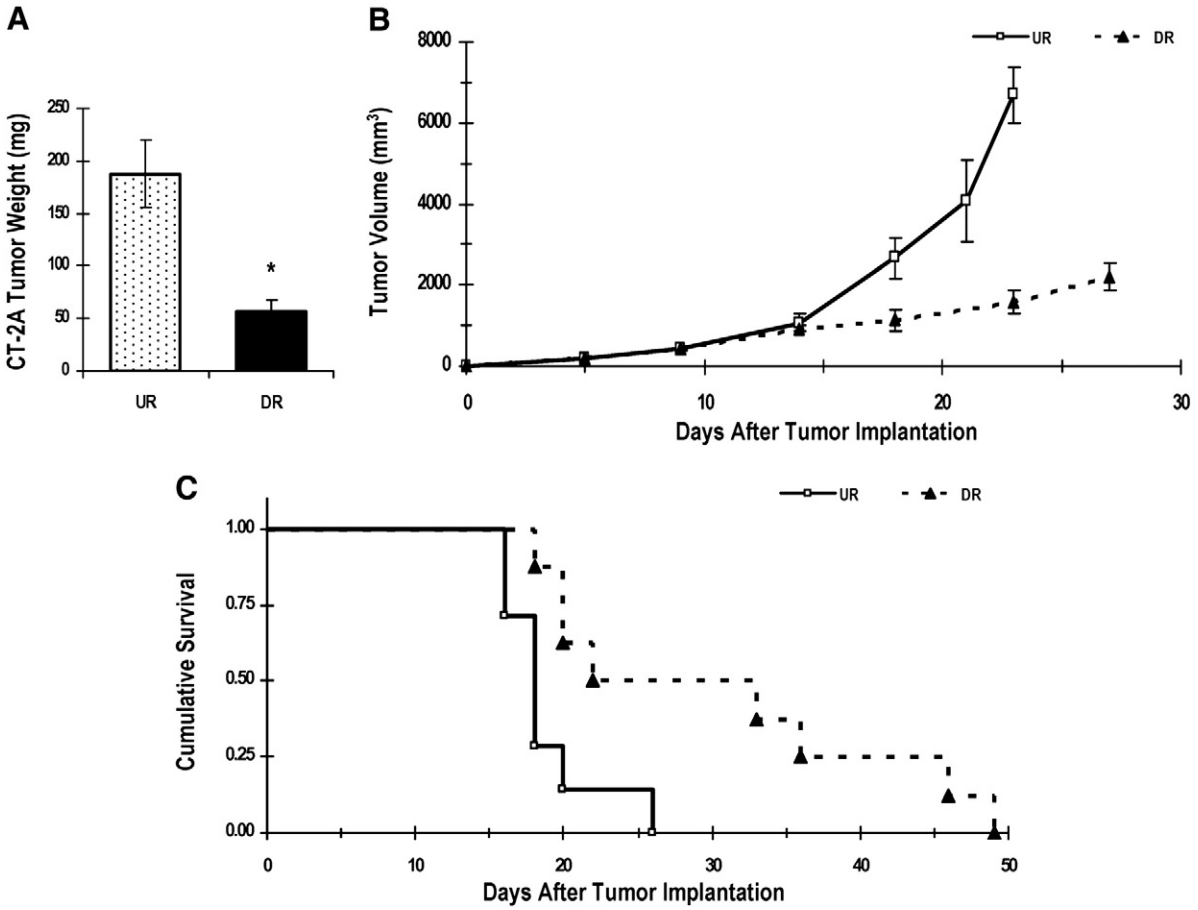

Fig. 3. Influence of DR on tumor growth and survival in mice bearing the CT-2A malignant astrocytoma. Intracerebral tumor weight (A); subcutaneous tumor volume (B); and Kaplan–Meier survival analysis (C). For (B, C), CT-2A tumor tissue was injected s.c. on day 0 and DR was initiated on day 14 when tumors were ~1000 mm^3 in volume. Subcutaneous CT-2A tumor volume was significantly lower from day 18 to day 22 ($P<0.01$; Student *t*-test), and mouse survival was significantly longer ($P=<0.01$; Kaplan–Meier survival analysis followed by Log-rank test) in the DR group than in the UR-fed control group. The asterisk indicates that average CT-2A tumor weight was significantly lower in the DR group than in the UR group at $^*P<0.005$ (Student *t*-test). Other conditions and details are as we previously described [85]. Reprinted from Clinical Cancer Research [85].

tumor in the VM inbred strain, is the only syngeneic mouse brain tumor to our knowledge that expresses the full complement of Scherer's secondary structures [253]. As seen in Fig. 5, CR reduced the growth and invasion of the VM-M3 primary tumor. Compared to the diffuse, ill-defined border of the VM-M3 tumor observed in the unrestricted control mice, the tumor grown in the CR mice appeared denser with a more defined border. CR also reduced the invasion of tumor cells from the implanted ipsilateral cerebral hemisphere into the contralateral hemisphere. While invading tumor cells were identified in all regions of the contralateral hemisphere of the control *ad libitum*-fed (AL) mice, only sub-pial invasion was found in the contralateral hemisphere of the CR group (Fig. 6). The total percentage of Ki-67-stained cells within the primary tumor and the total number of blood vessels was also significantly lower in the CR-treated mice than in the mice fed AL, indicating that CR is also anti-proliferative and anti-angiogenic in this tumor [253]. Our findings with CR therapy in the invasive VM-M3 glioblastoma model are in contrast to those observed with bevacizumab therapy, which appears to enhance glioma invasion without reducing Ki-67 positive tumor cells [41,42,254]. Our findings suggest that CR could be a more effective anti-angiogenic therapy than bevacizumab for brain cancer management. Also, the therapeutic efficacy of CR was not associated with diarrhea or other adverse effects, as occurs with the potent epidermal growth factor receptor (EGFR) inhibitor, gefitnib [38]. Although the molecular mechanisms by which CR reduces invasion are not yet fully described, these findings indicate that the anti-invasive properties of CR can be due in part to a reduction of proliferative, glycolytic, and angiogenic factors in both the tumor cells and in the tumor microenvironment.

11. Drug/diet synergy for managing malignant astrocytoma

Although dietary energy restriction is effective in reducing brain tumor growth and progression, this therapeutic approach alone is unlikely to completely eradicate all types of malignant brain tumors. We think that metabolic diet therapy could be enhanced when combined with drugs that also target energy metabolism. Support for this hypothesis comes from our recent pilot study showing that the non-metabolizable glycolysis inhibitor, 2-deoxy-D-glucose (2-DG), worked synergistically with the RKD to reduce CT-2A astrocytoma growth [189]. 2-DG is readily transported into cells, is phosphorylated by hexokinase, but cannot be metabolized further and thus accumulates in the cell [255]. This leads to ATP depletion and the induction of cell-death. In this regard, 2-DG has been described as a CR-mimetic, a drug that mimics some aspects of calorie restriction [256,257]. However, treatment of animal models and cancer patients with relatively high doses of 2-DG (greater than 200 mg/kg) was largely ineffective in managing tumor growth [258–260]. Side effects of 2-DG included elevated blood glucose levels, progressive weight loss with lethargy, and behavioral symptoms of hypoglycemia [258–262]. These findings indicate that 2-DG alone is ineffective as a viable therapy for most cancers.

Few studies have evaluated the therapeutic efficacy of anti-glycolytic or anti-cancer drugs in combination with restricted diets. Recent studies suggest that calorie restriction and fasting can enhance the therapeutic

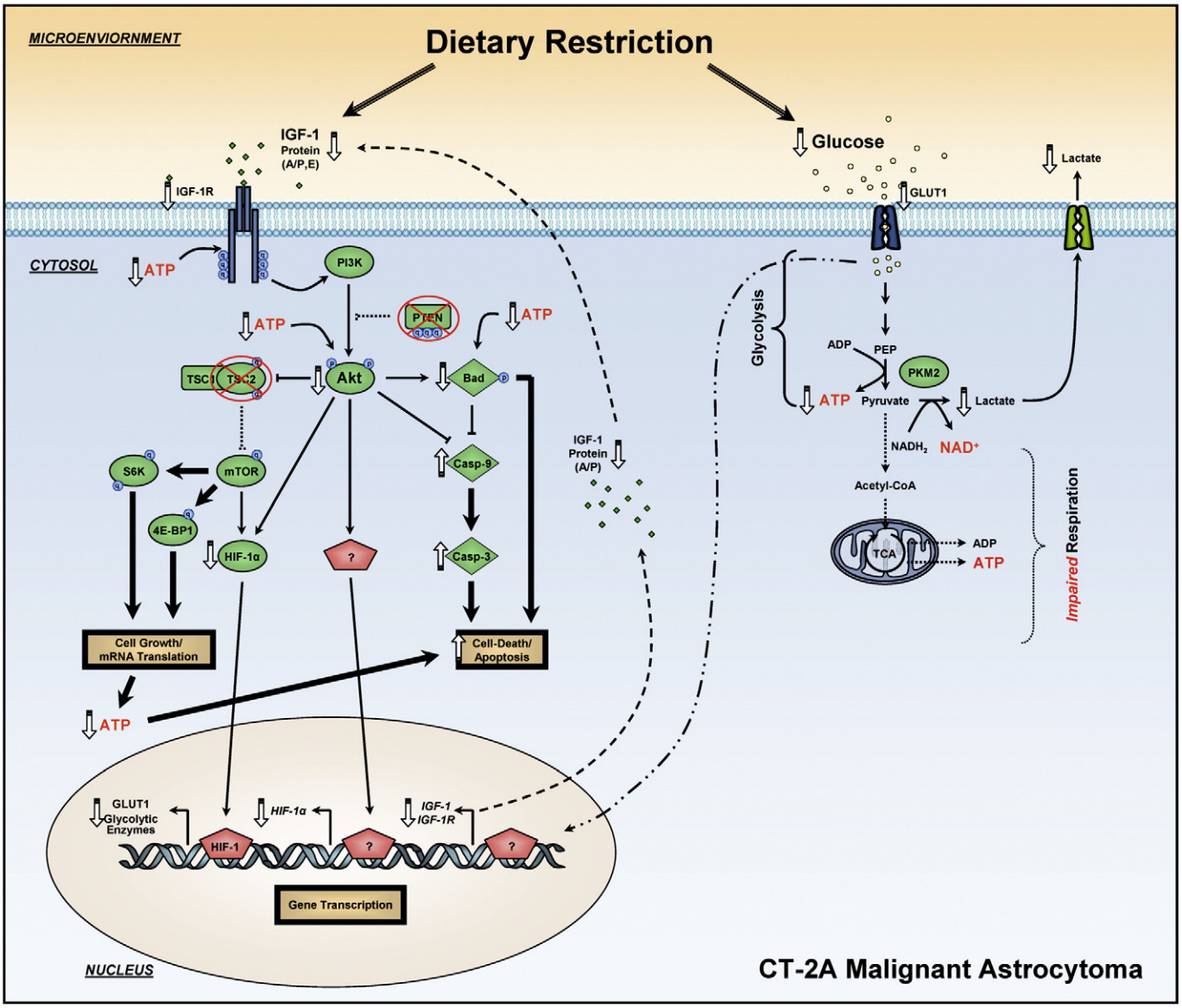

Fig. 4. Proposed mechanism by which late-onset DR acts as a broad-spectrum inhibitor of Akt signaling and growth in the PTEN/TSC2-deficient CT-2A astrocytomas. DR reduces glucose and IGF-1 (autocrine (A)/paracrine (P) and endocrine (E)) metabolism. Reduced production of IGF-1 will inhibit signaling through the IGF-1R/Akt pathway and lead to activation of apoptotic pathways induced by the dephosphorylation of BAD (on S-136) and cleavage of procaspase-9/-3. The expression of HIF-1α and GLUT1 are regulated in part by the level of Akt phosphorylation. Consequently, increased expression of HIF-1α and GLUT1 confer protection against apoptosis. The DR-induced suppression of Akt phosphorylation leads to reduced transcription and translation of HIF-1α as well as to decreased expression of GLUT1. We propose that the inhibition of glucose metabolism by DR plays a central role in mediating the antagonistic effects of DR in managing the metabolically inflexible PTEN/TSC2-deficient astrocytomas. In other words, the loss of PTEN and TSC2 expression in malignant astrocytomas could impair adaptation to energy stress produced by DR. Moreover, the inability of CT-2A to shutdown protein synthesis during DR–owing partially to loss of the PTEN and TSC2 tumor suppressors–may also contribute to DR-induced cell-death by accelerating ATP depletion. The shapes with green backgrounds represent signal transduction molecules in the cytosol whereas the pentagons with red backgrounds represent transcription factors. Upward facing arrows represent increased expression whereas downward facing arrows represent decreased expression. Question marks represent unknown transcription factors. Other conditions and details are as we previously described [85]. Reprinted with permission from Clinical Cancer Research [85].

action of anti-cancer drugs [263,264]. We showed that a low dose of 2-DG (25 mg/kg) was ineffective in reducing CT-2A astrocytoma growth, but that this same dosage had powerful anti-tumor effects when combined with the RKD [189] (Fig. 7). Indeed, the KD-R supplemented with a low dose of 2-DG was effective in reducing intracerebral tumor growth to a greater extent than was either 2-DG or the KD-R administered alone, indicating a synergistic interaction between the drug and the diet. We suggested that energy stress was greatest in the mice receiving the drug/diet combination [189]. Based on the findings from this study and from those of the Longo group [263,264], we suggest that the therapeutic efficacy of many anti-cancer drugs, which are marginally effective or toxic when administered alone, could be more effective when administered in combination with energy restricted diets. It is important to mention, however, that calorie or dietary restriction does not target glutamine, and might therefore be less effective in managing the growth of tumors that depend more on glutamine than on glucose [188]. Consequently, metabolic therapies that target both glucose and glutamine are likely to have the greatest therapeutic effect in managing tumor growth [48,188].

The findings in mouse brain tumors exemplify the efficacy and versatility of reduced calorie intake as a broad-spectrum inhibitor of malignant glioma growth and suggest that dietary energy restriction

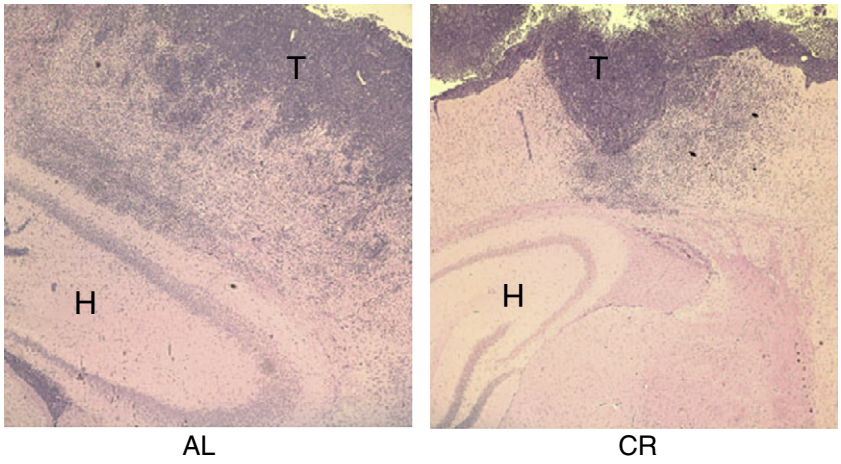

Fig. 5. Influence of calorie restriction on VM-M3/Fluc tumor growth. VM-M3/Fluc tumor fragments were implanted into the cerebral cortex fixed and were stained with haematoxylin and eosin (H&E) as described [253]. Images are shown at 50× (T = tumor, H = Hippocampus). At least 3 samples were examined per group. Reprinted with permission from ASN Neuro [253].

may extend survival in patients with advanced brain cancers because it simultaneously targets multiple metabolic pathways in tumor cells without causing adverse effects or toxicity to normal cells [84,85,253]. DR will facilitate ketone elevation while maintaining low normal glucose levels. The global energy transition from glucose to ketones will reduce inflammation in the tumor microenvironment thus reducing progression. Basically, dietary energy restriction and ketone body metabolism delays entropy [48,91]. Cancer is a disease of accelerated entropy [48,265]. This metabolic therapy could be even more effective when combined with drugs that also target energy metabolism. Hence metabolic therapies, which lower glucose availability and elevate ketone bodies, can reduce brain tumor growth through integrated anti-angiogenic, anti-invasive, and proapoptotic mechanisms.

12. Complicating issues for implementing metabolic therapy for malignant brain cancer

Several issues can complicate attempts to implement metabolic diet therapy for brain cancer management in patients. Availability of a drug that would mimic the global therapeutic effects of dietary energy restriction would certainly be the easiest way to implement the therapy. However, no drugs are known that can simultaneously lower glucose levels while elevating ketones in the absence of some form of calorie restriction, though the recently described ketone ester diets could be an exception [68]. Consequently, a major issue is the non-conventional and non-pharmacological nature of the metabolic therapy. Modern medicine has not looked favorably on diet therapies for managing complex diseases especially when well-established procedures for acceptable

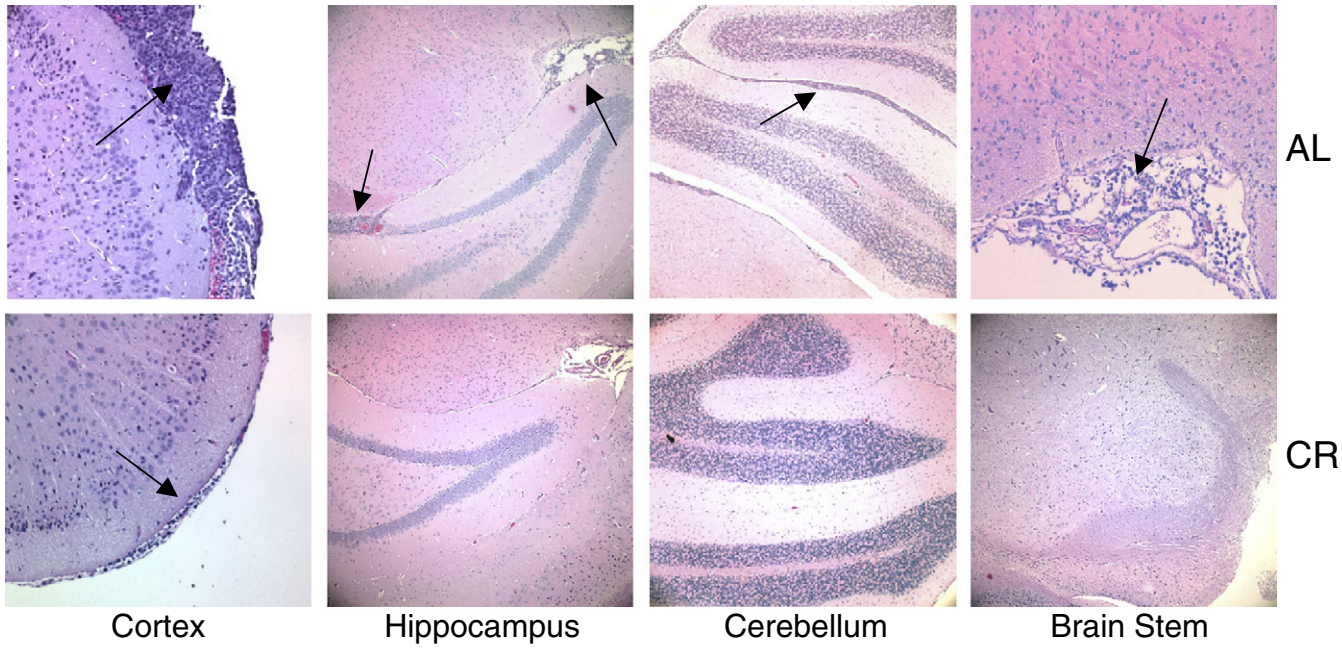

Fig. 6. Influence of calorie restriction on VM-M3/Fluc tumor cell invasion to the contralateral hemisphere. VM-M3/Fluc tumor fragments were implanted as described [253]. Histological analysis (H&E) was used to validate the presence of tumor cells under AL (top) and CR (bottom) in cerebral cortex (200×), hippocampus (100×), cerebellum (100×), and brain stem (200×). Arrows indicate the presence of tumor cells. At least 3 samples were examined per group. Reprinted with permission from ASN Neuro [253].

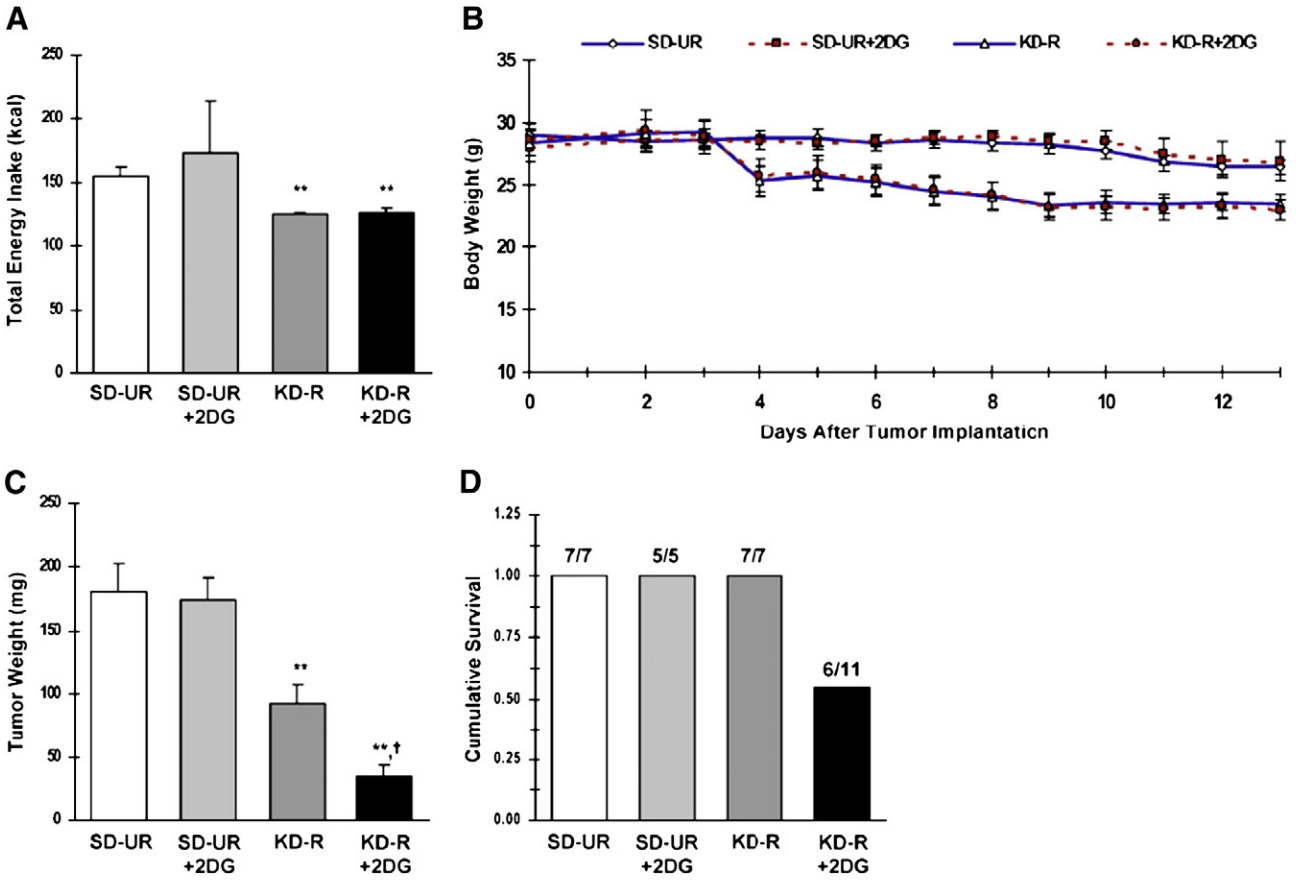

Fig. 7. Influence of the restricted ketogenic diet with or without 2-DG on total energy intake (A), body weight (B), tumor growth (C), and on cumulative survival (D) in mice bearing the orthotopically implanted CT-2A malignant astrocytoma. All mice were fed the standard high carbohydrate rodent diet in UR amounts for the first 3 days after tumor implantation prior to their separation into one of four diet groups ($n = 5$–11 mice/group) fed either SD-UR or a KD-R with or without 2-DG (25 mg/kg) for 10 days. The four groups were matched for body weight. 2-DG was initiated 6 days after tumor implantation and was continued for 7 days (B and C). As shown in (B), the feeding paradigm for the KD-R and KD-R+2- DG groups was designed to reduce body weights by ~20% relative to values recorded before the diet was initiated (3 days after tumor implantation). The average total energy intakes in (A) represent the number of kcals consumed by the indicated group over the dietary treatment period (day 3 to day 13). All values are expressed as the mean ± S.E.M. In (A and C), average values for the indicated group are significantly less than the average value for the SD-UR group at $^{**}P<0.01$. The mean value for the KD-R+2DG group is significantly lower than the mean value for the KD-R group at † $P<0.01$. No significant differences were observed between the SD-UR and SD-UR+2DG groups throughout the study. For (D), the number of tumor-bearing mice that were alive in each group at the conclusion of the study is listed as a ratio above each solid vertical bar (e.g. the "6/11" indicates that 6 of the 11 original mice were alive at the end of the study in the associated group). Reprinted with permission from Nutrition & Metabolism.

clinical practice are available, regardless of how ineffective these procedures might be in managing the disease [34]. In the case of brain cancer management, these approved practices generally involve maximal surgical resection followed a few weeks later by either radiation therapy or radiation and chemotherapy [10,12]. Some patients can also be subjected to multiple surgeries that do little to enhance long-term survival. Almost all patients receive corticosteroids, which significantly elevate blood glucose levels. The type of therapy given will usually depend on the age and health status of the patient. However, the number of older patients with glioblastoma that are either offered no therapy or who choose no therapy appears to be increasing [6]. Significant neurological damage can often occur in those children who survive malignant brain cancer while the risk of developing long-term morbidity and mortality is greatly enhanced [195,266–269]. Worse yet, some conventional therapeutic protocols involving combinatorial radiotherapy with chemotherapy or anti-angiogenic therapy may actually exacerbate the disease [35,41–43]. These situations are unacceptable and highlight the inadequacies of conventional approaches for malignant brain cancer management in either adults or children. Indeed, healthy long-term survivors of these conventional practices are more the exception than the rule.

Despite this bleak situation, the brain tumor field continues with expensive clinical trials using new combinations of radiation and/or toxic drug therapies in the hope of finding a therapeutic approach with improved efficacy [8,37,38]. We find it remarkable that so many brain cancer patients are recruited for therapies that are toxic, potentially lethal, and offer little hope for improved clinical outcome. Why does this situation persist? More than 60 years of clinical research indicates that such approaches are largely ineffective in extending survival or improving quality of life. Tragically, some of these therapies accelerate the demise of some brain cancer patients. This is especially the case for radiation with steroid therapy, which will elevate glucose and glutamine in the microenvironment thus enhancing metabolic vitality of surviving tumor cells [34]. Therapeutic approaches to brain cancer management, which produce adverse effects and reduce quality of life, should not be pursued, especially when more effective and less toxic alternative metabolic therapies are available. A recent study showed that the adverse effects of rash and diarrhea were correlated with very modest increase in survival of GBM patients [38]. Without appropriate control groups for rash and diarrhea, it is difficult to interpret such findings. As most brain cancer therapies are toxic to cells and tissues, toxicity has become the norm rather than the exception for new cancer therapies. The situation could change once the field comes to appreciate the nature of cancer as primarily a metabolic disease [48]. A problem is in recognizing the existence and scientific basis for effective, non-toxic, alternative metabolic approaches for brain cancer management.

How can effective non-toxic metabolic therapies be introduced as part of the standard clinical practice in the field? It is incumbent upon

neuro-oncologists to notify patients that effective alternatives to the current standards of care exist for managing brain tumors. Patients should also know that the RKD would retard tumor growth without producing toxic adverse effects. It should be up to the patient and their family to decide whether or not the RKD is a viable therapeutic option for their situation. Patients with malignant brain tumors, especially those with glioblastoma, should have the opportunity to compare and contrast the results from recent drug studies [38,40], with those of metabolic therapy using restricted diets [84,195]. While entrenched practices within the field might make it difficult for some physicians to suggest the RKD as a therapeutic option for brain cancer management, it is hoped that some enlightened physicians will come to recognize the effectiveness of the non-toxic metabolic approach to brain cancer management.

Another issue in implementing restricted diets for brain cancer management concerns the mechanism of action. How can the process of targeting glucose and glutamine, while elevating ketone bodies through dietary energy restriction, be so effective in managing malignant brain cancer? The process is rooted in the well-established scientific principle that tumor cells are largely dependent on substrate level phosphorylation for their survival and growth [48,62,76,85,177,195,270]. Glucose and glutamine drive substrate level phosphorylation. Because tumor cells are less flexible than normal cells in using alternative energy substrates (ketones), tumor cells will experience more energy stress when access to these fuels becomes restricted. While the concept might appear simple, the underlying mechanisms are the subject of considerable investigation and debate. This should not, however, retard initiation of clinical trials.

Another concern is how a metabolic therapy that reduces food intake and body weight can be recommended to patients who might be loosing body weight because of cancer cachexia [48]. Pro-cachexia molecules such as proteolysis-inducing factor are released from the tumor cells into the circulation and contribute to the cachexia phenotype [106,271,272]. By targeting the glycolytically active tumor cells that produce pro-cachexia molecules, restricted diet therapies can potentially reduce tumor cachexia [18,48,106]. Once the tumor becomes managed, patients can increase caloric consumption, which will accelerate weight gain. Hence, restricted consumption of ketogenic diets could be effective, in principle, for managing tumor growth in brain cancer patients with cachexia [195,271].

In contrast to most conventional brain tumor therapies, which expose both normal cells and tumor cells to toxic assaults, DR and particularly the RKD, are the only known therapies that can target brain tumor cells while enhancing the health and vitality of normal brain cells [19,62,84,195]. In this regard, restricted calorie intake is conceptually superior to most current conventional brain cancer therapies. Support for our position on this issue can be established through clinical trials for brain cancer patients similar to those trials conducted previously for epilepsy patients [273].

Another difficulty with calorically restricted diets for brain cancer management is the lack of a standardized use protocol for all patients. In other words, how is the diet implemented? This is a legitimate concern that hinders applicability to a broad range of patients, as most neuro-oncologists are unfamiliar with the application of metabolic therapy for brain cancer management. Similar concerns are often raised for implementing the ketogenic diet as a therapy for persons with epilepsy. Fortunately, several medical groups have established protocols and menus for implementing ketogenic diet or low glycemic diets in children [274–277]. Clinicians and could easily adapt these protocols and menus for their brain cancer patients. Nebeling and Lerner also provided a protocol for using the medium chain triglyceride ketogenic diet for brain cancer management [278].

The KD for management of epilepsy and brain cancer would be similar except in the degree of calorie restriction, which is somewhat greater for brain cancer patients than for epilepsy patients in order to more effectively reduce blood glucose levels. Since most reasonably healthy adults can tolerate more dietary restriction than can children, adults have greater flexibility than children in using calorically restricted diet therapies for brain cancer management. Children with brain tumors, however, respond well to the ketogenic diet as Nebeling and co-workers showed. According to Herbert Shelton, a guru on human fasting, most healthy adults can fast (water only) for up to 30 days without adverse effects [91,279]. The issue of patient compliance should not be a problem, as motivation for a potentially effective non-toxic therapy is generally enhanced when confronting a life-threatening disease like malignant brain cancer. If patients consider the "track record" of success and the excessive toxicity and financial expense of most conventional brain tumor therapies, motivation for using a RKD as an alternative therapeutic option should not be a problem [8]. Why are most patients not given this option?

13. Guidelines for implementing dietary management of malignant brain cancer

We suggest a sequential series of therapeutic phases for the dietary management of malignant brain cancer in patients. **Phase one** would gradually lower circulating glucose levels and elevate circulating β-OHB levels over a 10- to 14-day period using restricted ketogenic diets or therapeutic fasting [62,91]. Blood glucose ranges between 3.0 and 3.5 mM (55–65 mg/dl) and β-OHB ranges between 4 and 7 mM should be effective for tumor management. These values are well within normal physiological ranges of glucose and ketones in humans and will have anti-angiogenic and proapoptotic effects causing metabolic isolation of tumor cells and significant growth arrest. We refer to this state in mice as the zone of metabolic management (Fig. 8).

The importance of maintaining low blood glucose levels cannot be overemphasized. Indeed, elevated blood glucose levels accelerate disease progression in patients with gliomas [16,17]. We first showed that astrocytoma growth rate in mice was directly correlated with blood glucose levels, i.e., tumors grew faster under high circulating glucose conditions and slower when glucose levels were reduced [18]. These responses are almost entirely predicted based on the validity of Warburg cancer theory. Dietary or calorie restriction provides an effective means to maintain low blood glucose levels. It is important to recognize, however, that "more is not better" with respect to the ketogenic diet, as consumption of excessive amounts of the ketogenic diet will maintain high blood glucose levels thus causing accelerated tumor growth [18,62,88]. Consequently, this metabolic therapy will require

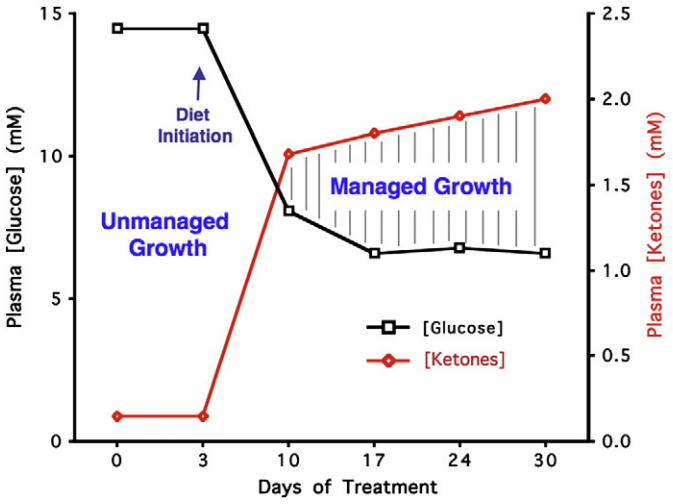

Fig. 8. Relationship of circulating glucose and ketone body levels to brain tumor management. These values are within normal physiological ranges of glucose and ketones under fasting conditions in mice and produce anti-angiogenic and proapoptotic effects causing metabolic isolation of tumor cells and delayed tumor growth. We refer to this state as the zone of metabolic management. The glucose and ketone levels predicted for brain tumor management in human patients are 3.1–3.8 mM and 2.5–7.0 mM, respectively [19]. (Reprinted with permission from *Epilepsia*, 49(Suppl. 8):114–116, 2008).

considerable personal discipline as water-only fasting will occasionally be required to lower glucose levels and to elevate ketone body levels in order to reach the therapeutic zone for managing tumor growth (Fig. 8).

The RKD can reduce the feeling of hunger while maintaining low glucose and elevated ketone body levels. A recent study in rats suggests that diets supplemented with ketone esters might have a similar effect, but this has not yet been tested in humans [68]. Glucose levels can be monitored several times/day with any standard glucose meter, while blood ketone levels can be monitored several times/week with either blood glucose/ketone meters or with enzyme assay as we described [63]. It is necessary to measure ketone levels in blood rather than in urine, as urine values may not reflect actual levels in the blood [280,281]. Evidence in mice clearly shows that blood ketone levels are higher when the KD is administered in restricted amounts than when administered in unrestricted amounts [64]. It is also imperative that brain tumor patients keep accurate daily records of their blood glucose levels and weekly records of their ketone levels. These biomarker data can be used in conjunction with tumor imaging data to better define the parameters for the zone of brain tumor management [84]. Dietary supplements of vitamins and minerals should not be a problem as long as their consumption does not elevate circulating blood glucose levels or reduce ketone levels. Flexibility in ketogenic and low glycemic food choices is possible as long as blood glucose and ketones can be maintained within the therapeutic range (Fig. 8).

Brain tumor imaging analysis can be used to periodically assess the efficacy of the diet therapy on tumor progression [8,84]. Tumor imaging using PET could be a problem, however, especially if the diet reduces glucose uptake. This would actually be a favorable outcome and suggestive of diet efficacy. Additionally, restricted ketogenic diets would reduce the need for antiepileptic drugs or steroidal medications for reasons described above. We discourage the use of high dosage steroid medication for brain cancer patients, as dexamethasone increases gluconeogenesis and blood glucose levels while enhancing apoptosis resistance in tumor cells [14,34,48,84,270]. While steroids can rapidly mitigate some aspects of the brain tumor phenotype over the short term (paralysis, edema, etc), high dose steroidal use will ultimately accelerate brain tumor recurrence and the demise of patients. Hence the RKD therapy, though not as fast acting as dexamethasone, will not harm patients as can high-dose dexamethasone.

Phase two of the therapy would involve surgical resection. We suggest surgical resection as an option after first implementing the restricted diet therapy. This option will only be possible if there is an opportunity for a "watchful waiting" period prior to scheduled surgery [282]. The option will not be possible, for those patients in a critical condition at the time of presentation. The diet will reduce tumor vascularization, progression, and will more clearly delineate tumor tissue from surrounding normal brain tissue as shown in Fig. 5. This can be assessed through MR and PET imaging [84,195]. Neurosurgeons should recognize that smaller brain tumors with reduced vascularity and clearly circumscribed boundaries should be easier to resect than larger brain tumors with poorly circumscribed boundaries and extensive vascularization. This would also ensure greater debulking thereby increasing the likelihood of long-term survival. The standard practice of surgical resection as soon as possible after tumor diagnosis could be counter productive for some patients, especially for those with lower-grade gliomas. The metabolic diet therapy will target angiogenesis and slow tumor progression naturally thus providing more time to consider the surgical option. The diet could also be implemented before surgical resection for some GBM patients, as surgical resection alone can alter the microenvironment thus enhancing the invasive behavior of tumor cells [33]. It is possible that progression free survival could be significantly extended in some GBM patients if an aggressive metabolic therapy were implemented prior to surgery. The urge to debulk malignant brain tumors as soon as possible after diagnosis may not be in the best interests of all patients and could actually exacerbate disease progression in some patients.

Finally, **phase three** could involve carefully executed diet cycling strategies to maintain metabolic pressure on surviving tumor cells [19,84,283]. Diet cycling for humans could include weekly transitions from calorically restricted ketogenic diets to nutritious low calorie, low glycemic diets. Like the ketogenic diet, low glycemic diets have also been used to manage seizures in children [284]. The input of board certified nutritionists would be helpful in guiding patients during these dietary transitions. An interesting therapeutic strategy could also involve low doses of glycolysis inhibitors combined with the RKD. We recently demonstrated a synergistic interaction between the glycolysis inhibitor 2-deoxyglucose (under low dosage) and the RKD for brain tumor growth inhibition [189]. We propose that diet/drug cocktail therapies may be even more effective for long-term brain cancer management than either therapy alone. Using this approach, we believe that ketone bodies could protect normal cells from the adverse effects of low glucose and ROS while effectively targeting the energy metabolism of the tumor cells. We are aware of several patients (both children and adults) who are presently using the RKD for brain cancer management with considerable success in retarding tumor growth.

14. Conclusions

We provide information on a new, alternative approach to brain cancer management using metabolic therapy with restricted ketogenic diets. The objective of this new therapeutic approach is to change the metabolic environment of the tumor and the host. Only those cells with a normal flexible genome, honed through millions of years of environmental forcing and variability selection, are expected to survive extreme shifts in metabolic environment [19]. Indeed, extreme conditions of survival and fitness will test the limits of a cell population's persistence in any given location over time [73,74]. Extensive genomic damage, as exists in most tumor cells, will reduce fitness under nutritional stress. We suggest that this therapeutic approach, illustrated with restricted diets, will be more efficacious than current approaches for brain cancer management because it is based on the principles of evolutionary biology, metabolic control theory, and the Warburg theory of cancer. While most of the research supporting our findings and recommendations has been conducted in animal models of brain cancer and in a few patients, we think that humans with brain cancer will respond better than the mice to this metabolic therapy. This comes from the work of Cahill and Veech showing that humans are possibly the most capable animal species in transitioning from glucose to ketone bodies for survival under fasting [89,96]. Support for our contention, however, must await clinical trials where patients have lowered their glucose levels and elevated their ketones levels to the recommended metabolic zone of therapeutic efficacy in the absence of radiation or toxic drug therapies. While the use of this metabolic approach to brain cancer management is presently not part of current medical practice in the field, we are hopeful that oncologists and patients will come to recognize the value of global metabolic transition for longer-term management of malignant brain tumors.

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