The Ketogenic Diet for the Treatment of Malignant Glioma

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Abstract

Advances in our understanding of glioma biology has led to an increase in targeted therapies in preclinical and clinical trials; however, cellular heterogeneity often precludes the targeted molecules from being found on all glioma cells, thus reducing the efficacy of these treatments. In contrast, one trait shared by virtually all tumor cells is altered (dysregulated) metabolism. Tumor cells have an increased reliance on glucose, suggesting that treatments affecting cellular metabolism may be an effective method to improve current therapies. Indeed, metabolism has been a focus of cancer research in the last few years, as many pathways long associated with tumor growth have been found to intersect metabolic pathways in the cell. The ketogenic diet (high fat, low carbohydrate and protein), caloric restriction, and fasting all cause a metabolic change – specifically, a reduction in blood glucose and an increase in blood ketones. We, and others, have demonstrated that these metabolic changes improve survival in animal models of malignant gliomas and can potentiate the anti-tumor effect of chemotherapies and radiation treatment. In this review we discuss the use of metabolic alteration for the treatment of malignant brain tumors.

Key words: metabolism, cancer, brain tumor, ketones, therapy, caloric restriction, glioblastoma multiforme, glucose, Warburg Effect

Brain Tumors:

Glioblastoma multiforme (GBM), the most aggressive type of brain tumor, represents one of the greatest challenges in the management of cancer patients worldwide. Despite aggressive surgery followed by radiation and chemotherapy, patients with newly diagnosed GBM have an average life expectancy of 12-18 months and less than 10% survive 5 years (1, 2). Brain tumors are highly infiltrative, and surgery rarely removes all tumor cells, particularly from eloquent areas of the brain. Further, while radiation and chemotherapy can kill *most* of the remaining tumor cells, those that survive typically regrow. Thus, these tumors often recur within 2 years of their original diagnosis and in the same general area as the primary tumor. The proximity of the recurrent tumor to the primary tumor often precludes the use of additional standard radiation therapy because of toxicity concerns (3). Once a GBM recurs following chemotherapy with temozolomide (TMZ), there are few additional chemotherapeutic agents with demonstrated efficacy for these tumors. The identification of new therapeutic targets for malignant gliomas has focused on molecular targets, often those found through global analyses done by The Cancer Genome Atlas consortium (4) and other groups (5-8). This work demonstrated that there are approximately four molecular subtypes of GBMs based on genetic aberrations, gene expression profiling and protein expression. One ultimate goal of these studies is the identification of therapeutic targets and a better understanding of how to determine the best patients for these specific targeted agents (9). However, solid tumors are heterogeneous, and these targets are typically not found on all cells in a tumor. The failure to successfully manage primary GBM and its recurrence remains a major challenge, and advances in survival and quality of life rely on new therapeutic approaches.

Tumor Metabolism:

Cancer cells must meet the demands of rapid proliferation, thus cellular energy metabolism is one of the main processes affected during the transition from normal to cancer cells. Otto Warburg first described this shift in what we now call aerobic glycolysis or the "Warburg Effect" in 1924 (10, 11). The Warburg Effect describes the tumor cell's use of glycolysis to provide energy and biomolecules regardless of the availability of oxygen. Under adequate oxygenation, normal cells rely on mitochondrial oxidative phosphorylation to generate ATP and switch to the less favorable anaerobic pathway of glycolysis when exposed to hypoxia. However, many types of cancer cells survive and proliferate by generating ATP via glycolysis

rather than oxidative phosphorylation even when oxygenated; a process that occurs very early in tumorigenesis, prior to hypoxia (12). We now know that cancer metabolism is much more complex than just a higher rate of glycolysis. Mitochondrial biogenesis is also altered, and the cancer cell's fate becomes reliant on the balance between the availability of energy, sufficient macromolecular synthesis for increased growth, and the modulation of reactive oxygen species (ROS)(13, 14).

Since Warburg's discovery, metabolism has been of interest in the cancer field, but it often seemed overshadowed by discoveries of oncogenes, tumor suppressor genes, growth factor pathways, molecular subtypes of cancers, etc. There is a resurgence of interest in metabolism as a central theme in cancer, and we continue to find that metabolic pathways intersect and often regulate key components of tumor initiation, progression and therapy response (15, 16). Many pathways long known to be associated with tumor cell growth, escape from apoptosis, aggressive blood vessel formation (angiogenesis) and therapy resistance have now been linked to cellular metabolism (17). For example, p53 is a tumor suppressor encoded by *TP53* which is frequently mutated in cancer. P53 promotes a variety of cellular responses to hypoxia, DNA damage and oncogene activation; however recently it has been found to regulate glycolysis and assist in maintaining mitochondrial integrity (18). The overactivation of the stress responsive PI3K/AKT signaling pathway, typical in many cancers, has also been closely linked to metabolism and under low glucose conditions results in rapid tumor cell death (17, 19, 20). Hypoxia, a common occurrence in the tumor microenvironment, induces hypoxia inducible factor 1 (HIF-1), which regulates the uptake of glucose and the expression of a number of genes involved in glycolysis and energy metabolism (21). Myc, an oncogene long known to be involved in malignant cell transformation has also been shown to play a role in metabolic regulation, particularly in response to changes in the tumor microenvironment (22). These connections, and others, suggest that targeting metabolic changes can and should be considered in the context of other, more classic therapeutic targets.

The Ketogenic Diet (KD)

The ketogenic diet (KD) is a medically regimented, high-fat low protein/carbohydrate diet used to treat refractory pediatric epilepsy (23, 24). It simulates fasting, thus increasing ketones and decreasing glucose in the blood, leading to high rates of fatty acid oxidation and an increase in the production of acetyl-CoA. When the amount of acetyl-CoA exceeds the capacity of the tricarboxylic acid cycle to utilize it, there is an increase in the production of the ketone

bodies β–hydroxybutyrate (βHB) and acetoacetate (ACA) which can be used as an energy source in the brain (25-28). While the mechanisms are not fully understood, the neuroprotective effects of a KD on the brain have led to interest in using it for the treatment of a host of neurological disorders including Alzheimer's disease, traumatic brain injury and amyotrophic lateral sclerosis (29, 30).

It has been postulated that the KD may be useful in exploiting tumor metabolism and the Warburg Effect. Unlike normal brain cells, many tumor cells cannot utilize ketones effectively due to their various genetic and mitochondrial defects, and must rely on glucose as their primary energy source (14, 31-36). Ketone bodies may also be toxic to some human tumor cells (37-39). So by reducing the glucose availability to cancer cells and providing ketones as an alternative energy source for normal cells, the KD may offer an approach to targeting the Warburg Effect in highly glycolytic tumors, such as malignant gliomas. However, as we have learned primarily from the epilepsy literature, the action of the KD is more complex and its anti-tumor actions are likely to extend beyond the effects of reduced blood glucose. For example, the KD has been shown to reduce reactive oxygen species (ROS) production in the brain (23, 40). ROS are multi-faceted effector molecules involved in numerous cellular pathways, including those regulating autophagic/apoptotic responses to genotoxic stress, inflammation, hypoxia and nutrient deprivation. Cancer cells often have increased levels of ROS (41) and they have been implicated in angiogenesis induction and tumor growth through the regulation of vascular endothelial growth factor (VEGF) and HIF-1 (42).

The KD and Caloric Restriction in Preclinical Studies

Early attempts to use metabolism as a therapeutic target often focused on caloric restriction (CR). In 1914, Payton Rous was the first to suggest that restricted food intake reduced tumor growth by reducing the tumor blood supply (43). More recently it has been suggested that CR reduces growth and angiogenic biomarker expression in prostate cancer and breast cancer (44-47). The combination of caloric restriction and the ketogenic diet (restricted ketogenic diet; RKD) has also been studied as a cancer therapy. The anti-proliferative and anti-angiogenic effects of CR and the RKD have been substantiated in a number of studies using experimental mouse and human brain tumor models (14, 32, 48-51). Angiogenesis is currently a therapeutic target for the treatment of gliomas, and bevacizumab, a monoclonal antibody targeting the proangiogenic factor, vascular endothelial growth factor (VEGF), is the only FDA-approved molecular drug for use in glioblastomas multiforme (GBMs). Unfortunately, this drug

often results in adverse effects and only a limited improvement in survival (52). If metabolic therapy could be used to provide a less toxic way to limit VEGF activity it may mimic the beneficial effects of bevacizumab with fewer side effects.

Tumors not only stimulate rapid angiogenesis but these blood vessels often develop immaturely and become very leaky (53). This can lead to an increased influx of inflammatory cells and signaling that can actually perpetuate tumor growth and damage surrounding normal tissue. A byproduct of this inflammation is the buildup of fluid around the tumor, or peritumoral edema, which is a frequent cause of morbidity and mortality in patients with gliomas. Dexamethasone is the current treatment of choice for peritumoral inflammation and edema, yet it comes with adverse side effects such as hyperglycemia, cardiovascular effects, osteoporosis, weight gain, insomnia, infection and cognitive effects which ultimately reduce the quality of life for patients (54, 55). Evidence suggests that CR and the RKD alters inflammatory pathways, normalizes vasculature and may reduce peritumoral edema. In a mouse astrocytoma model, CR reduced expression of pro-inflammatory markers, cyclooxygenase-2 (COX-2), nuclear factor kappa B (NF-κB) and macrophage inflammatory protein (MIP-2) (56). Seyfried and colleagues recently showed that CR promoted vessel maturation by preventing vascular VEGF signaling in the CT-2A mouse astrocytoma model (57) Further, a recent study in the U87 human glioma model showed that CR normalized a variety of factors involved in tumor vessel instability and leakiness, including VEGF, and showed a reduction of peritumoral edema (51).

While CR and RKD can easily be administered in animal models of malignant tumors and there is anecdotal evidence and a few case reports of efficacy in humans, there has been resistance in the medical community to use CR for cancer patients. An alternative strategy is the unrestricted KD (UKD) which has a long safety record for the treatment of pediatric epilepsy and may be easier for patients to maintain (58). Thus, this approach may meet with less resistance from the clinical community. In the case of the UKD, “unrestricted” is something of a misnomer, as caloric sources and overall caloric intake are carefully monitored by a registered dietician to attain appropriate ketone and glucose levels.

Recent studies have shown that an unrestricted ketogenic diet (UKD) also causes a reduction in blood glucose, an elevation of blood ketones and extends life in an immunocompetent mouse model of malignant glioma (59, 60). In the GL261 mouse glioma model, the UKD decreased tumor vasculature, reduced peritumoral edema, and altered the expression of genes involved in angiogenesis (Woolf et al., manuscript in preparation), paralleling the CR results mentioned above. Results also demonstrated that increasing blood ketones affects a number of tumor-related gene networks. This includes alteration in the

expression of genes involved in the cellular response to oxidative stress in tumor tissue, notably COX-2, leading to a reduction in reactive oxygen species (ROS) (59). A separate study using the same model found that the UKD plus radiation therapy also reduced expression of both COX-2 and Nf-κB while reducing the production of ROS (61). Additional changes in gene expression suggest that the UKD may inhibit insulin-like growth factor (IGF-1), platelet-derived growth factor (PDGF) and epidermal growth factor receptor (EGFR) signaling pathways (39) as has been shown in various CR and RKD studies (49, 51, 57).

The KD not only targets specific aspects of tumor biology as described above, but may also exert a global effect on the aberrant genetic landscape found in tumors. We have used cDNA Array technology to demonstrate that overall gene expression in tumor from animals fed the UKD was shifted more towards the gene expression found in non-tumor containing tissue from animals fed either the UKD or standard diet (59). While the mechanism(s) through which this global shift in gene expression as a result of the UKD is not known, one hypothesis is that the UKD may be altering the tumor epigenome. The epigenome describes the collection of abnormal, heritable changes in gene activity that are not caused by changes in the DNA sequence (62). These modifications include chromatin remodeling, histone modifications, DNA methylation, and microRNA pathways; all of which have now been linked to metabolism in many cancers, including brain tumors (63, 64). Epigenetic changes in cancer are now being looked at as potential therapeutic targets. New therapies such as histone deacytelase (HDAC) inhibitors are actively being tested for their ability to reverse the abnormal gene expression patterns inherent to the cancer epigenome and for their ability to enhance other anti-tumor therapies (65, 66). A recent study suggests that β-hydroxybutyrate (βHB), the major ketone that is increased in the blood in animals and patients on the UKD can alter the epigenetic landscape in mammalian cells by inhibiting HDAC (67). Another study showed that the UKD reversed the major epigenetic modifications found in the brains of epileptic rats (68). While the effect of the KD on tumor epigenetics has yet to be studied directly, evidence warrants further exploration.

KD as an Adjuvant Therapy

While the studies described above show that the KD and/or CR provides various anti-tumor benefits on their own, evidence suggests that they may also enhance other therapies for brain tumors by either protecting normal tissue, working in synergy with other treatments, or both. Gene expression changes in the tumors from animals fed the UKD were not the same as those in the non-tumor containing contralateral side of the brain (39, 59). This allows for the

hypothesis that the neuro-protective activity of blood ketones may also function to reduce the deleterious side effect of cranial radiation on normal brain. A recent publication showed that fasting, which elevates blood ketones, not only sensitizes many types of cancer cells to standard therapies but may promote the protection of normal tissue from the toxicity associated with radiation and chemotherapy (69).

As an adjuvant to radiation and chemotherapy, the UKD has demonstrated a large synergistic effect. The UKD greatly enhanced survival in a mouse model of glioma when combined with TMZ when compared to either treatment alone (70). In addition, a separate study showed that 9 out of 11 animals maintained on the UKD and treated with radiation had complete and sustained remission of their implanted tumors, even after being switched back to a standard rodent diet (60). Another recent study found that combining the KD with radiation and chemotherapy resulted in decreased tumor growth rate and increased survival in a lung cancer xenograft model (71). Studies also show that CR and fasting may act in synergy with other anti-cancer therapeutics (69, 72-75).

It has been suggested that the KD and/or caloric restriction may be more effective in combination with other agents targeting metabolism and specifically glucose. It has been shown that a RKD given in combination with the glycolysis inhibitor, 2-deoxy-D-glucose (2DG), reduced the growth of a mouse CT-2A astrocytoma to a greater extent than either therapy administered alone (76). Metformin, a therapy for diabetes mellitus, and the analog phenformin are becoming a focus in the cancer metabolism research community due to their antitumor activity in a variety of *in vitro* and *in vivo* cancer models, including brain tumors (77); however they have not been investigated in combination with CR or the KD. Hyperbaric oxygen is another experimental anti-cancer therapy that works by reversing tumor hypoxia, which can contribute to a tumor's dependence on glycolysis (78) and it has recently been demonstrated that the UKD showed a synergistic effect when used with hyperbaric oxygen therapy, prolonging survival in a mouse model of metastatic cancer over either therapy alone (79).

KD In Humans

The first use of the KD for the treatment of human malignant brain tumors was in 1995 by Nebeling and colleagues. The patients in this study were two female children diagnosed with nonresectable advanced stage brain tumors (anaplastic astrocytoma stage IV and cerebellar astrocytoma stage III), both of which had undergone extensive radiation and chemotherapy. The goal of the study was to determine if dietary induced ketosis could decrease the availability of

glucose to disrupt tumor metabolism while maintaining the nutritional status of the patients. Both children responded remarkably well to the KD, showing a reduction in glucose uptake and experiencing long-term tumor management (80).

In 2010, researchers in Italy published a case report on 65-year-old female patient with multicentric glioblastoma multiforme (GBM) that was treated with a restricted calorie ketogenic diet (RKD) during standard radiation and chemotherapy. The patient followed the 4:1 (ratio of fats:carbohydrate plus protein) ketogenic diet restricted to 600kcal/day which resulted in reduced levels of blood glucose and elevated levels of urinary ketones. After two months on the diet, the patient's body weight was reduced by about 20%, however most importantly; no observable brain tumor was detected using either Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) or magnetic resonance imaging (MRI). Ten weeks after stopping the diet, the tumor recurred and CPT11 (Irinotecan) and bevacizumab chemotherapy was initiated (81). The patient succumbed to her disease less than 2 years after diagnosis. Nevertheless, this work demonstrated that the RKD could be tolerated in a brain tumor patient, at least for a short period of time, and it appeared to have some efficacy. In 2011, German researchers evaluated the restricted-calorie KD in 16 subjects with various types of advanced cancers who had exhausted all therapeutic options. This pilot trial showed that the KD did not cause any severe side effects or changes in cholesterol or blood lipids. Of the 16 subjects, 5 were able to complete the 3-month treatment period and none of these patients experienced further tumor progression while on the diet (82). Two of the 11 remaining patients died early following the beginning of the trial, one was unable to tolerate the diet and dropped out immediately, 2 patients dropped out for personal reasons, one was unable to continue the diet for more than a month and 3 had disease progression within less than 2 months of starting the diet and one dropped out to resume chemotherapy. On the whole, this pilot study demonstrated that the KD could be tolerated in some patients with advanced disease and it appeared to be beneficial in 5 of the 16 subjects; however, the overall results were hard to interpret based on the variety and severity of disease in the enrolled patients.

More recently, a number of prospective clinical trials have been initiated. A study in Germany is evaluating the efficacy of a calorie-restricted ketogenic diet and transient fasting during re-irradiation for patients with recurrent GBM (NCT01754350). Michigan State University is directing a similar trial evaluating a calorie-restricted KD for the management of recurrent GBM (NCT01535911). A third pilot study is evaluating the KD as adjunctive treatment in refractory/end-stage GBM (NCT01865162). The goals for all of these studies are to obtain data on the safety, efficacy and tolerability of the KD as an adjunctive therapy for patients with GBM.

The only study using the KD as an up-front, concurrent therapy has recently been approved and is now open for enrollment at St. Joseph's Hospital and Medical Center and Barrow Neurological Institute in Phoenix, Arizona (ClinicalTrials.gov identifier pending). This trial for patients with primary GBM will evaluate the classic 4:1 ketogenic diet therapy during radiation treatment and concurrent temozolomide followed by the modified Atkins Diet (1:1 fat:carbohydrate plus protein) during temozolomide treatment.

While the KD holds promise as an anti-cancer therapy, clinical utilization is not without its challenges. More data is needed to define the optimum "therapeutic range" for blood glucose and ketone levels, and to determine if varying formulations of the KD would be more effective in different individuals. Further studies are also needed to determine the necessary duration and long term effects of the KD. A limited number of papers suggest that long term use of the UKD may in fact have deleterious effects including glucose intolerance in rats (83-85). However, it should be noted that the composition of the diet is critical, and the ongoing support of a registered dietician well versed in its use can reduce the likelihood of adverse effects in humans. In addition, the medical community must be educated on the therapeutic value of metabolic alteration as an adjuvant therapy, even if it results in a small amount of healthy weight loss, since the current dogma is to avoid weight loss in patients undergoing chemotherapy for fear of increased fatigue and further decline in overall patient health. In fact, it has been suggested that a high fat diet may even reduce cachexic weight loss, a source of reduced overall health in cancer patients (86). As with any clinical decision, implementation of therapy must be guided by the assessment of the patient's individual situation, which should include nutritional status. Quality of life is also a concern as this type of nutritional therapy requires discipline, motivation and careful guidance by a registered dietician experienced in implementing the KD. Compliance can be made more difficult by the use of steroids (prescribed for peritumoral edema) that often increase hunger and raise blood glucose levels. Despite these caveats, the existing preclinical data suggesting anti-tumor efficacy and a synergistic effect with standard therapies provides a strong impetus to conduct controlled clinical trials, particularly those that will shed light on the interactions between the KD and other therapies.

Conclusions

The anti-tumor mechanisms through which the ketogenic diet, caloric restriction (and intermittent fasting) and other potential metabolic therapies act are not completely understood; however, the animal model data strongly suggest that metabolic alteration may be a highly

effective therapy as well as a potent adjuvant to the current standard of care for malignant brain tumors. The KD and/or CR are the only therapeutic approaches that simultaneously target multiple hallmarks of cancer such as energy metabolism, angiogenesis, and inflammation. This suggests a number of avenues for further research such as: (i) Can we mimic the effects of some current chemotherapies using metabolic alteration; (ii) will the use of the KD provide an effective way to reduce the confounding effects of tumor heterogeneity by targeting the abnormal metabolic processes underlying brain tumors and many other cancers; (iii) can the neuro-protective actions of metabolic therapies that increase blood ketones help reduce the deleterious side effects of current therapies and (iv) can the use of standard therapies be augmented by altering the cancer's intrinsically aberrant cellular metabolism? These and other questions can only be answered using carefully constructed clinical trials that include metabolic alteration. Our increased understanding of metabolism as both a driving hallmark of cancer and an important therapeutic strategy provides important insight and suggest the KD may be an effective way to enhance the way brain tumors are treated.

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