Chapter 15

Anti-Angiogenic and Pro-Apoptotic Effects of Dietary Restriction in Experimental Brain Cancer: Role of Glucose and Ketone Bodies

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Abstract: Angiogenesis involves neovascularization or the formation of new capillaries from existing blood vessels and is associated with the processes of tissue inflammation, wound healing, and tumorigenesis. Recent studies from this laboratory show that moderate restriction of dietary energy intake (dietary restriction, DR) has powerful anti-angiogenic and pro-apoptotic effects against the CT-2A experimental mouse astrocytoma. DR reduces blood glucose levels while elevating ketone bodies. As most brain tumour cells are dependent on glycolysis for energy due to mitochondrial defects, they are unable to switch from glucose to ketone bodies for energy. An energy source shift from glucose to ketone bodies will enhance the bioenergetic potential of normal brain cells while reducing tumour cell growth and tissue inflammation. It is suggested that cancer therapeutics that reduce tumour growth, while also reducing food intake and body weight, may operate in large part through the anti-angiogenic and pro-apoptotic effects of DR.

Key words: Astrocytoma, caloric restriction, cell death, IGF-1, inflammation, vascularity, angiogenesis, apoptosis, metabolic control theory, brain cancer, CT-2A management, anti-angiogenic therapies, ketogenic diet

1. INTRODUCTION

The long-term prognosis remains poor for most patients with malignant brain tumours despite advances in the molecular genetics of cancer and in brain imaging techniques (1, 2). Surgical resection followed by radiation is the standard therapy today as it has been for over five decades. Chemotherapy also has had little positive benefit on malignant glioma management and is often associated with adverse effects that diminish quality of life (1, 3). It is also unlikely that therapeutic targeting of tumour-associated mutations will be effective in brain tumour management, as most tumour mutations arise as epiphenomena of tissue disorganization and their involvement with tumour initiation, promotion, or progression has not been conclusively established (4-7). Clearly, alternative therapies are needed that can better manage brain tumours while permitting a decent quality of life.

2. BRAIN TUMOUR ANGIOGENESIS

Angiogenesis involves neovascularization or the formation of new capillaries from existing blood vessels and is associated with the processes of tissue inflammation, wound healing, and tumorigenesis (8-10). A significant literature suggests that vascularity is rate limiting for the formation of solid tumours including brain tumours (10-15). The malignancy and invasiveness of tumours are also correlated with the degree of their vascularity since prognosis is generally better for tumours that are less vascular than for those that are more vascular (15-18). The inhibition of vascularity is therefore considered an

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important therapeutic strategy for controlling tumour growth (14, 19-26).

Factors that influence the migration and proliferation of endothelial cells may underlie the mechanisms of angiogenesis. Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen that is a reliable biomarker of angiogenesis in human brain tumours (16, 20, 27-30). Besides VEGF, several of other growth factors and cytokines act as angiogenesis inducers (8, 31, 32). Elevated IGF-1 levels are associated with enhanced brain tumour progression and angiogenesis (33-35). Indeed, reduced IGF-1 levels are associated with reduced angiogenesis and increased apoptosis (36, 37). In general, the degree of tumour vascularity reflects a relative balance between angiogenesis inducers and inhibitors (24, 38).

Tumour progression is thought to involve a change in the balance of angiogenesis inducers over inhibitors (24). The inducers and inhibitors of angiogenesis may originate from both the tumour cells and from tumour-infiltrating host cells, e.g., endothelial cells and macrophages (8, 9, 39-41). Anti-angiogenic therapies may therefore influence brain tumour growth through effects on these cells. We recently suggested that dietary energy restriction may shift the microenvironment of brain tumours from a pro-angiogenic to an anti-angiogenic state through multiple effects on the tumour cells and on the tumour associated host cells (42).

3. DIETARY RESTRICTION AND CANCER

Dietary restriction (DR) is produced from a total restriction of dietary nutrients and differs from starvation in that DR reduces total caloric energy intake without causing anorexia or malnutrition (36, 43-47). In 1914, Rous first suggested that underfeeding might inhibit mouse tumour growth by delaying tumour vascularity (angiogenesis) from the host (48). Later studies showed that the anti-tumour effects of DR resulted from caloric restriction *per se* and not from the restriction of any specific dietary component such as proteins, vitamins, minerals, fats, or carbohydrates (36, 43, 44, 49). In addition to tumour growth inhibition, DR also produces a marked increase in general health consistent with the notion that *ad libitum* feeding of sedentary rodents or humans is overfeeding (44, 45, 50). Although the restriction of dietary energy intake (caloric restriction) underlies the anti-tumour effects and health benefits of DR, the molecular mechanisms for these phenomena have not been clearly described.

4. ANTI-ANGIOGENIC AND PRO-APOPTOTIC EFFECTS OF DR IN AN EXPERIMENTAL ASTROCYTOMA

4.1 Brain Tumour Model

In our initial study, we investigated the effects of DR on the orthotopic growth and angiogenic properties of the experimental mouse astrocytoma, CT-2A (42). This syngeneic brain tumour was generated in our laboratory after implantation of 20-methylcholanthrene into the cerebral cortex of a C57BL/6J mouse according to the procedure of Zimmerman (51, 52). Histologically, the CT-2A brain tumour is broadly classified as a poorly differentiated highly malignant anaplastic astrocytoma (51). The tumour grows orthotopically as a soft, noncohesive, and highly vascularized mass.

4.2 Intracerebral tumour implantation

We implanted the CT-2A tumour into the cerebral cortex of C57BL/6 mice using a trocar as previously described (53, 54). Small CT-2A tumour pieces (about 1 mm^3) from a C57BL/6J donor mouse were used for the cerebral implants. We prefer the initiation of brain tumours from intact tumour pieces rather than from cultured cells since the pieces already contain an established microenvironment that facilitates tumour growth. Moreover, tumours initiated from tissue pieces more closely match the natural tumour environment and do not require adaptation from an unnatural cell culture environment.

4.3 Dietary restriction

Prior to the initiation of our experiments, we separated and randomly assigned mice to either a control group that was fed AL (*ad libitum*) or to an experimental group that was fed a total DR of 30% (70% of the control group) (42). Each mouse was housed singly and was given a cotton-nesting pad for warmth. In our initial experiments, DR was initiated 7 days prior to tumour implantation and was continued for either 11 or 14 days after implantation (42). Total DR maintains a constant ratio of nutrients to energy, i.e., the average daily food intake (grams) for the AL fed mice was determined every other day and the DR-fed mice were given 70% of that quantity on a daily basis (36). All mice received PROLAB RMH 3000 chow (Purina, LabDiet, Richmond, IN), which contained a balance of mouse nutritional ingredients and, according to the manufacture's specification, delivered about 4.4 Kcal/g gross metabolizable energy. Body weights of all mice were recorded every other day.

4.4 Tumour growth

We analyzed intracerebral tumour growth directly by measuring total tumour dry weight. Tumours were dissected from normal appearing brain tissue, were frozen, and then lyophilized to remove water. From our experience, total tumour dry weight is a more accurate measure of CT-2A tumour growth than total wet weight because individual tumours can vary in the degree of hemorrhage and edema.

4.5 Influence or DR on CT-2A Growth, Angiogenesis, and Apoptosis

We found that CT-2A brain tumour growth was about 80% less under moderate DR than under AL feeding (Figure 1). This reduction in tumour growth greatly exceeded the 12% reduction in body weight during the 22-day experiment. Several previous studies showed that moderate DR could reduce the growth of histologically diverse non-neural tumours (36, 43, 46, 48). Our studies are the first to document this phenomenon in a brain tumour model and suggest that brain tumours are especially vulnerable to the growth-inhibitory effects of DR. We have since documented this phenomenon in other mouse and human brain tumour models (109).

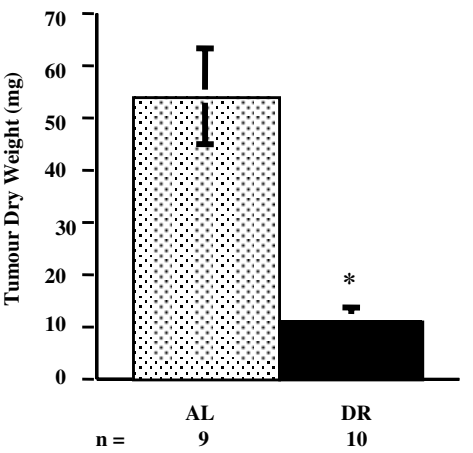

Figure 1. Influence of DR on the intracerebral growth of the CT-2A brain tumour. DR was initiated 7 days before tumour implantation and was continued for 14 days after implantation. Values are expressed as means + SEM and n = the number of tumour-bearing mice examined in each group. The asterisk indicates that the dry weight of the treated tumours was significantly lower than that of the control tumours ($P < 0.001$, two tailed t-test). (with permission from BJC).

The DR-induced reduction in CT-2A growth was also associated with significant reduction in the number and size of blood vessels and with significant elevation in TUNEL positive cells (apoptosis) (Table 1 and Figure 2). In other words, we found that DR is both anti-angiogenic and pro-apoptotic in this brain tumour model. These effects also occurred without causing significant reductions in CT-2A cell proliferation as assessed using the proliferating cell nuclear antigen (PCNA) assay (42). Other investigators have also reported that anti-angiogenic growth factors and cytokines can reduce tumour microvessel density, increase apoptosis, but have little effect on cell proliferation (55-58). Our

results therefore support previous findings that DR produces a pattern of biomarker changes similar to the changes seen following anti-angiogenic therapies (36, 59).

Table 1. Effects of dietary restriction on microvessel density, apoptosis, and proliferation index in the CT-2A brain tumour.

Treatment [a]	Microvessel density [b] vessels/high-power field	Apoptotic index % [c]	Proliferation index % [d]
AL	27.3 ± 3.9	3.8 ± 0.9	71.5 ± 5.8
DR	13.0 ± 2.0 *	9.9 ± 0.6 **	69.7 ± 4.9

[a] Animals were fed either *ad libitum* (AL) or under dietary restriction (DR) as described in Text. Three independent tumours chosen at random were analyzed in each group and all values are expressed as means ± SEM. The asterisks indicate that the values from the DR group differed from AL group at $P < 0.05$ *, and $P < 0.01$ ** as determined by the two tailed t-test.

[b] Factor VIII- positive microvessels were averaged in three hotspot areas of each tumour section at 200 x.

[c] Apoptotic index, determined from the TUNEL assay, 400x.

[d] Proliferation index, determined from the PCNA assay, 400x. (with permission from BJC)

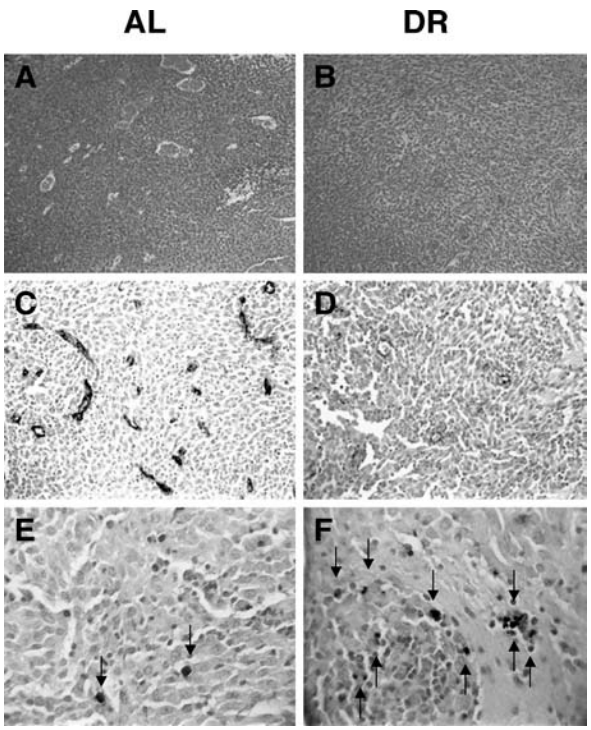

Figure 2. Influence of DR on microvessel density and apoptosis in the CT-2A brain tumour. DR was initiated 7 days before intracerebral tumour implantation and was continued for 11 days. H & E stained tumour sections in an AL mouse (A) and in a DR mouse (B) (100 x). Factor VIII immunostaining from the tumour grown in an AL mouse (C) and in a DR mouse (D) (200 x). TUNEL positive apoptotic cells (arrows) from the tumour grown in an AL mouse (E) and in a DR mouse (F) (400 x). Each stained section was representative of the entire tumour. All images were produced from digital photography. (with permission from BJC)

4.6 Implications of DR for Anti-Angiogenic Therapeutics

Our findings with the CT-2A brain tumour are relevant to those *in vivo* studies where food intake and body weight are reduced in conjunction with anti-angiogenic or anti-cancer therapies. For example, if a new anti-angiogenic drug reduces both body weight and tumour growth in experimental test subjects, it is necessary for the investigators to demonstrate the extent to which the angiogenic effect is due specifically to the drug and not to DR. Tannenbaum and Mukherjee previously mentioned that tumour therapies, which secondarily restrict food intake or assimilation, may produce changes in tumour growth that could be mistaken for a primary effect (44, 59). Recent studies also indicate that many anti-tumour drugs may also have 'accidental' anti-angiogenic effects (60). We suggest that cancer therapeutics that reduce tumour growth, while also reducing food intake and body weight, may operate in large part through the anti-angiogenic and pro-apoptotic effects of DR.

The inclusion of both pair-fed controls and active body weight controls in the analysis of new experimental drugs could help distinguish the anti-angiogenic and pro-apoptotic effects of the drug from that of DR. We recently found that complete starvation of mice for two days was necessary in order for an active body weight control group to match the weight loss in mice injected i.p. with temozolomide (100 mg/kg) (Mukherjee and Seyfried, unpublished observation). As some drugs may reduce food assimilation, active body weight controls must be evaluated together with pair-fed controls. Unfortunately, many scientific reports of new anti-angiogenic drugs fail to include all of the necessary control groups needed to distinguish

specific from nonspecific effects. It is our contention that few if any systemic anti-angiogenic brain tumour therapies will be as effective as DR in reducing angiogenesis.

5. INFLUENCE OF GLUCOSE AND KETONE BODIES ON CT-2A TUMOUR GROWTH

5.1 Brain Tumours Lack Metabolic Versatility

Reductions in plasma levels of glucose and elevations in ketone bodies (acetoacetate and β-hydroxybutyrate) are key biomarker changes associated with DR (49, 61). In contrast to normal brain neurons and glia, that can metabolize ketone bodies for energy when blood glucose levels decrease as occurs during fasting or caloric restriction (47, 61-63), gliomas and most tumour cells lack this metabolic versatility and are largely dependent on glycolytic energy (64-68). Defects in ketone body metabolism, the mitochondrial TCA cycle, and electron transport chain systems are thought to underlie the dependence of tumour cells on glycolytic energy (69-73). Hence, therapies that exploit the genetic and metabolic weakness of brain tumour cells should be effective in controlling brain cancer.

5.2 Ketogenic Diet Management of Pediatric Astrocytoma

Support for the concept that brain tumours are vulnerable to metabolic stress came in 1995, when Nebeling and coworkers reported that a ketogenic diet (KD) could manage advanced stage malignant astrocytoma in two female pediatric patients (74). The KD is a high fat, low protein, low carbohydrate diet that has been used for decades to treat patients with refractory epilepsies (47, 75, 76). It was not clear, however, whether the KD controlled pediatric astrocytoma through effects on plasma glucose or ketone bodies since the diet was administered under restricted conditions where blood glucose levels were also reduced (74). Although the findings with pediatric astrocytoma generated considerable interest in the brain tumour field (77), no further studies were conducted in humans to evaluate the anti-tumour effects of the KD. The reason for this is not clear since one of the patients is still alive and well at the time of this writing (Nebeling, personal communication). Clearly, further studies are warranted on the use of the KD and other diet therapies for brain cancer management.

5.3 Influence of Diet on CT-2A Tumour Growth and on Circulating Levels of Glucose and Ketone bodies

As a follow-up to the Nebeling study, we evaluated the efficacy of the KD in the CT-2A mouse astrocytoma. To determine if the content or composition of dietary calories was responsible for tumour growth inhibition, we compared the effects of the low carbohydrate, high fat KD with a high carbohydrate, low fat standard (SD) diet under both restricted and unrestricted feeding conditions (49). The nutritional composition of the two diets is shown in Table 2. After tumour implantation as before, we randomly assigned the mice to one of four diet groups that received either: 1) the standard diet fed *ad libitum* or unrestricted (SD-UR), 2) the KD fed *ad libitum* or unrestricted (KD-UR), 3) the SD restricted to 40% (SD-R), and 4) the KD restricted to 40% of the control standard diet (KD-R). The average daily food intake (grams) for the UR fed mice was determined every other day and the R-fed mice were given 60% of the SD-UR group amount on a daily basis. This ensured that the mice in both R mouse groups received a similar number of total calories throughout the study. The dietary treatments were initiated 24 hours following tumour implantation and were continued for 13 days. The study was terminated at this time to avoid the stress of tumour burden. We also recorded body weights of all mice every other day.

Table 2. Composition (%) of the standard diet (SD) and the ketogenic diet (KD)

Components	SD [a]	KD [b]
Fat (F)	6	75
Protein (P)	27	14
Carbohydrates (C)	62	0
Fiber	5	12
Kcal/g	4.4	7.8
F/P + C	0.07	5.4

[a] Standard diet was obtained from Purina, LabDiet, Richmond, IN.

[b] Ketogenic diet was obtained from Zeigler Bros. Inc., Gardners, PA.

The CT-2A tumour grew rapidly and to a similar large size in both groups of UR-fed mice (Figure 3). Restricted feeding, of either the SD or the KD, significantly reduced tumour growth. The R-fed mice shown in Figure 3A were representative of those mice with the largest tumours in their respective groups. The UR-fed mice, however, were not representative of those with the largest tumours. Tumour dry weights were approximately 74 % less in both R-fed groups than in their respective UR-fed control groups (Figure 3B). The reduction in tumour growth exceeded the 12-15% reduction in final body weight in the R-fed groups (49). All implanted tumours grew in both the UR-fed and R-fed groups suggesting that restricted feeding of either the SD or the KD did not prevent tumour "take" or establishment, but significantly reduced the intracerebral growth of the malignant CT-2A brain

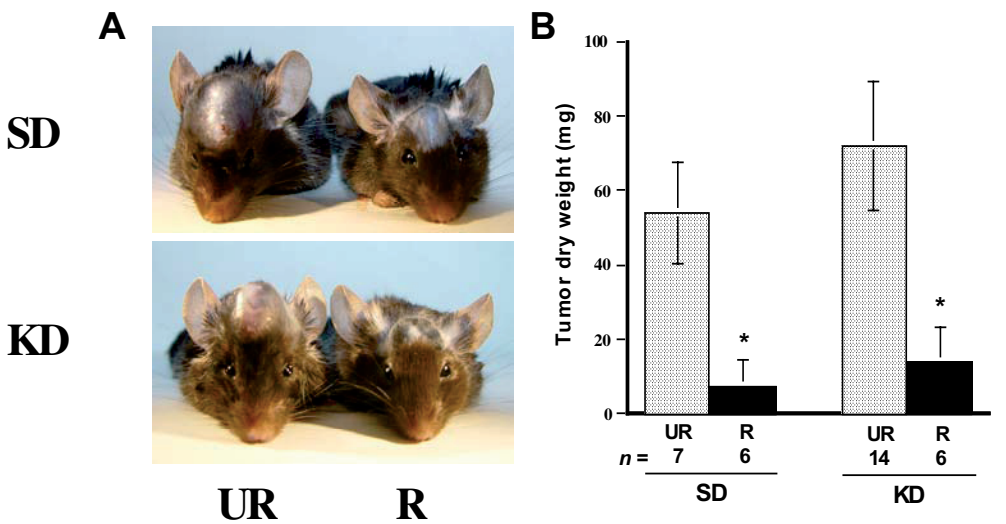

Figure 3. Influence of diet on the intracerebral growth of the CT-2A brain tumour. Dietary treatment was initiated 1 day after tumour implantation and was continued for 13 days. The visual representation (A) and quantitative assessment (B) of tumour growth in C57BL/6J mice receiving either the standard diet (SD) or ketogenic diet (KD) under either unrestricted (UR) or restricted (R) feeding. 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 tumours in R groups were significantly lower than those in the UR groups at $P < 0.01$.

CT-2A growth reduction was associated with reduced blood glucose levels (Table 3). We used linear regression analysis to show that blood glucose levels could predict CT-2A growth (Figure 4) (49). Although blood ketone levels were elevated under restriction of either diet, elevated ketone levels alone could not account for reduced tumour growth because tumour growth was rapid in the UR-KD group despite the presence of high ketone levels (Table 3). These findings support the previous observations of Fearon and co-workers who showed that the failure of a KD to restrict growth of the

Walker 256 rat tumour resulted from the failure of ketosis to reduce glucose availability (78). This is also consistent with our previous findings that blood glucose levels remain high in epileptic mice that consume the KD ad libitum and do not lose body weight (75). We suggest that reduced blood glucose may have contributed to the management of advanced stage malignant astrocytoma with the KD used in the Nebeling study (74, 77). Hence, reduced glucose, associated with reduced caloric intake, is a key factor in the metabolic control of the mouse CT-2A tumour and also possibly for the human pediatric astrocytomas in the Nebeling study.

Table 3. Influence of diet on plasma glucose, ß-OHB, and IGF-1 levels in mice bearing the CT-2A intracerebral brain tumour[a]

Diet[b]	Groups[c]	Glucose (mmol/L)	ß -OHB (mmol /L)	IGF-1 (ng/ml)
SD	UR	9.1 ± 0.9 (7) [d]	0.6 ± 0.1 (7)	208 ± 25 (6)
	R	5.2 ± 1.1* (6)	1.4 ± 0.2* (6)	117 ± 36* (6)
KD	UR	11.4 ± 1.4 (14)	1.0 ± 0.3 (14)	294 ± 30 (5)
	R	5.7 ± 1.5* (6)	1.3 ± 0.6 (6)	193 ± 57* (6)

[a] Values are expressed as means + 95% confidence intervals.
[b] Animals were fed either a standard chow diet (SD) or a ketogenic diet (KD).
[c] UR (unrestricted feeding) and R (restricted to 60% of the SD-UR group as described in Text).
[d] Numbers in parentheses indicate the number of independent tumor-bearing mice examined in each group.
The asterisks indicate that the values of the R groups differed from those of their respective UR groups at $P < 0.01$ (analyzed by ANOVA, one way) (with permission from BJC)

In contrast to the situation with prostate cancer and other non-neural cancers (36, 43, 79, 80), little is known about the influence of diet on the progression of brain cancer. We found that orthotopic growth of the CT-2A brain tumour was similarly rapid during the unrestricted feeding of either a high carbohydrate, low fat SD or a high-fat, low carbohydrate KD. On the other hand, CT-2A growth was significantly reduced when either diet was restricted to 60% of the control diet. These findings indicate that orthotopic CT-2A brain tumour growth, like prostate tumour growth, is influenced more by the amount of dietary calories than by the origin or source of the calories (36, 43). Hence, diet and lifestyle may influence the progression of brain cancer.

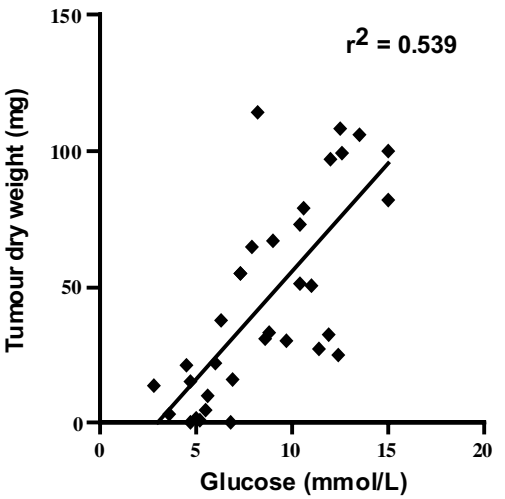

Figure 4. Linear regression analysis of plasma glucose and CT-2A-tumour growth in mice from both the SD and KD dietary groups combined (n = 34). These analyses included the values for plasma glucose and tumour growth of individual mice from both the UR and R-fed groups. The linear regression was highly significant at $P < 0.001$. (with permission from BJC)

5.4 Influence of Restricted Diets on Plasma IGF-1 levels in CT-2A Tumour Mice

As with glucose, we found that circulating IGF-1 levels were significantly lower in each R-fed mouse group than in the respective UR-fed group (Table 3). Linear regression analysis also showed that plasma glucose is predictive of plasma IGF-1 levels (49). These observations agree with previous findings that glucose regulates IGF-1 expression (81, 82). Since reduced IGF-1 levels are associated with reduced angiogenesis and increased apoptosis (36, 37), our findings provide further evidence that DR is anti-angiogenic and pro-apoptotic and that either blood glucose or IGF-1 levels may be useful biomarkers for predicting the effects of DR on brain tumour growth and angiogenesis (49).

6. METABOLIC CONTROL THEORY AND BRAIN CANCER MANAGEMENT

Metabolic control theory applies principles of bioenergetics for the control or management of complex diseases (47, 83, 84). Since metabolism is a universal process underlying all phenotypes, modification of metabolism can potentially modify phenotype. The theory is based on the idea that compensatory genetic and biochemical pathways regulate the bioenergetic potential of glycolysis, the tricarboxylic acid (TCA) cycle, and the electron transport chain. This produces a flexible and versatile metabolic system that is capable of restoring an orderly adaptive behavior to widely disordered conditions involving complex gene-environmental interactions (47, 83, 85). As biological chaos underlies the progression of brain tumours (4), principles of metabolic control theory may be effective in managing brain cancer.

We suggest that accumulated tumour mutations restrict the metabolic versatility of CT-2A tumour cells. As DR inhibits glycolysis, DR would force the CT-2A cells to switch from glucose to alternative non-carbohydrate energy metabolites, e.g., ketone bodies (47, 49). While this switch occurs readily in normal cells, the switch should be more difficult for the tumour cells due to their genetic defects (49). Regardless of whether the genetic defects arise as a cause or consequence of tumour growth, they will to some degree restrict metabolic flexibility. DR would therefore produce catastrophic energy failure and apoptosis in those CT-2A cells that lack metabolic flexibility and are solely dependent on glycolysis. Recent findings also showed that the glycolysis inhibitor 2-deoxy-D-glucose (2DG) or glucose deprivation enhances apoptosis through caspase-3 activation and PARP cleavage in human breast and lung cancer cells (86-88). Since DR reduces blood glucose levels, it is possible that the DR-induced apoptosis in the CT-2A tumour occurs through similar glucose-dependent caspase-3 apoptotic pathways. Our most recent findigs support this hypothesis (109).

We found that DR alone is incapable of killing all CT-2A tumour cells since the tumours continue to grow, though slowly, despite persistently reduced glucose and elevated ketone body levels (Mukherjee and Seyfried, unpublished). The survival of some tumour cells under the metabolic stress of DR may result in part from DR-enhanced gluconeogenesis (89). The liver and kidney are largely involved in gluconeogenesis during calorie restriction. It is also likely that the major glucose transporter, GLUT-1, is up-regulated in CT-2A cells following reductions in circulating glucose levels. Glycerol, released through hydrolysis of triacylglycerol and converted to glucose, will also contribute to circulating glucose levels that can be used by the tumour cells. These physiological adaptations to DR could provide just enough energy to maintain the survival and growth of CT-2A tumour cells. Nevertheless, the CT-2A tumour cells under DR are weakened and likely susceptible to additional forms of metabolic stress.

6.1 Role of Ketone Bodies in CT-2A Management

If the anti-tumour effects of restricted caloric intake are associated with reduced glucose levels and glycolytic energy, a question arises as to what role elevated ketone levels might have in CT-2A management. We suggest that ketone body metabolism, while providing normal brain cells with an alternative high-energy substrate, also reduces the inflammatory activities of tumour-associated host cells (stromal cells) (49). Ketone body metabolism reduces oxygen free radicals, enhances tolerance to hypoxia, and may prevent organ dysfunction from inflammatory processes (84, 90-93). Indeed, Dong et al reported that moderate calorie restriction could reduce the proinflammatory properties of macrophages while enhancing their phagocytic function (94). This is important since activated macrophages contribute to tumour angiogenesis (39, 95). Macrophages secrete numerous pro-angiogenic factors including VEGF, and the degree of tumour angiogenesis is generally associated with the number of macrophages (8, 9, 11, 17, 38-40, 94, 96-99). Uncoupling the detrimental inflammatory activities of macrophages from their potentially beneficial phagocytic activities is considered important for the eventual management of brain cancer (4). Hence, a shift in energy metabolism from glucose to ketone bodies will enhance the bioenergetic potential of

normal brain cells on the one hand while reducing tumour cell growth and tumour inflammatory properties on the other hand.

The key to controlling brain cancer will depend to a large extent on the combined effects of lowering glucose availability while increasing ketone availability. Our findings show, however, that ketone elevation alone is incapable of reducing brain tumour growth unless glucose is also reduced. This indicates that the brain tumour cells will continue to metabolize glucose for energy despite the presence of elevated ketones. Although brain tumour cells may take up ketone bodies, ketone bodies cannot be metabolized for energy if the mitochondria are defective. Roeder and coworkers showed that cultured brain tumour cells use glucose for energy and ketones for lipid synthesis (100). It is our contention that experimental brain tumour management may be achieved if glucose levels can be lowered to maximally stress the glycolytic-dependent tumour cells while providing enough ketone bodies to satisfy the energy needs of normal brain cells.

7. IS DR A PRACTICAL ANTI-ANGIOGENIC AND PRO-APOPTOTIC THERAPY FOR BRAIN CANCER IN THE CLINIC?

The pioneering studies of Nebeling and co-workers using the ketogenic diet to treat pediatric astrocytoma suggests that pediatric brain cancer can be managed with diet therapies that reduce glucose and elevate ketone bodies (74, 77). It is yet unclear if DR can produce anti-angiogenic and pro-apoptotic effects in human brain tumours similar to those we found in the CT-2A mouse astrocytoma. We think this may not be the case since basal metabolic rate is significantly less in humans than in mice (101). The anti-angiogenic and pro-apoptotic effects of moderate DR may therefore be less in human brain tumours than in mouse brain tumours.

In contrast to mice, however, adult humans are capable of complete fasting for prolonged periods with minimal adverse effects (47). We, therefore, speculate that a total food fast in adult humans will produce the physiological conditions of moderate DR in mice. This comes from recent findings that a total food fast lowers blood glucose and IGF-1 levels while elevating blood ketone body levels in healthy non-obese humans (102). Moreover, periodic fasting is known to improve general health to include reduction of tumour growth (103). Fasting will also elevate circulating glucocorticoid levels that will further reduce tumour angiogenesis and edema (104, 105). Glucocorticoids restrict glucose availability to tumour cells and thus will enhance tumour cell apoptosis and reduce angiogenesis (106). Although the synthetic glucocorticoid, dexamethasone, is often administered to brain tumour patients, severe adverse effects are associated with the long-term use of this compound (107). A total food fast in adult humans with brain tumours may, therefore, produce anti-angiogenic and pro-apoptotic effects similar to those that we found in DR mice bearing the CT-2A astrocytoma. For chilhood brain tumors, on the other hand, the pediatric dietary protocol of Nebeling may be most effective for tumour management (77).

A frequent criticism of the use of fasting or DR as a therapy for brain cancer comes from the misconception that voluntary food restriction (anorexia) may exacerbate patient weight loss from tumour-associated cachexia. In other words, how can fasting be justified as an anti-angiogenic/pro-apoptotic brain tumour therapy if the patient is already loosing weight from the tumour? Weight loss associated with cancer cachexia, however, differs from weight loss associated with anorexia since cachexia can occur without anorexia and is produced from factors actively released by the tumour (108). We suggest that fasting or DR may antagonize cachexia by reducing tumour size and thereby reducing the levels of pro-cachexic factors. Although appearing counterintuitive, fasting may facilitate patient weight gain once the fast is broken. The timing of the fast is another critical variable for use as a potential brain tumour therapy. We suggest that the therapeutic benefit of fasting will be best when initiated soon after brain tumour diagnosis or surgical resection, i.e., at a time when normal brain cells can easily switch from glucose to ketone body metabolism. This energy switch may be more difficult following radiation or chemotherapies that

reduce the physiological health of normal brain cells and may actually contribute to brain tumour progression (4).

In summary, we suggest that identification of the anti-angiogenic and pro-apoptotic mechanisms of DR in experimental mouse and human brain tumour models will facilitate translation of this diet therapy to the clinic. It is interesting that an enormous effort is presently underway in the pharmaceutical industry to identify new cancer drugs with anti-angiogenic and pro-apoptotic effects. Since DR already produces these effects, in addition to improving general physiological health, it is surprising that a greater research emphasis is not devoted to this area. We suggest that deciphering the molecular and biochemical mechanisms by which DR reduces angiogenesis and enhances apoptosis may produce new brain tumor drugs that are more effective and biologically friendly than those currently available.

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REFERENCES

1. Shapiro, W. R., 1999, Arch Neurol, 56:429-32.
2. McLendon, R. E, and Halperin, E. C., 2003, Cancer, 98:1745-8.
3. Yung, W. K., Albright, R. E., Olson, J., Fredericks, R., Fink, K., Prados, M. D., Brada, M., Spence, A., Hohl, R. J., Shapiro, W., Glantz, M., Greenberg, H., Selker, R. G., Vick, N. A., Rampling, R., Friedman, H., Phillips, P., Bruner, J., Yue, N., Osoba, D., Zaknoen, S., and Levin, V. A., 2000, Br J Cancer, 83:588-593.
4. Seyfried, T. N, 2001, Perspect Biol Med, 44:263-82.
5. Sonnenschein, C., and Soto, A. M., 1999, The Society of Cells: Cancer and the Control of Cell Proliferation. Springer-Verlag, New York.
6. Sonnenschein, C., and Soto, A.M., 2000, Mol Carcinog, 29:205-11.
7. Bergstein, I., 2003, Mol Carcinog, 36:1-5.
8. Sunderkotter, C., Steinbrink, K., Goebeler, M., Bhardwaj, R., and Sorg, C., 1994, J Leukoc Biol, 55:410-422.
9. Folkman, J., 1992, Semin Cancer Biol, 3:65-71.
10. Jendraschak, E., and Sage, E.H., 1996, Semin Cancer Biol, 7:139-146.
11. Nishie, A., Ono, M., Shono, T., Fukushi, J., Otsubo, M., Onoue, H., Ito, Y., Inamura, T., Ikezaki, K., Fukui, M., Iwaki, T., and Kuwano, M., 1999, Clin Cancer Res, 5:1107-13.
12. Assimakopoulou, M., Sotiropoulou Bonikou, G., Maraziotis, T., Papadakis, N., and Varakis, I., 1997, Anticancer Res, 17:4747-53.
13. Wesseling, P., Ruiter, D. J., and Burger, P. C., 1997, J Neurooncol, 32:253-265.
14. Kim, K.J., Li, B., Winer, J., Armanini, M., Gillett, N., Phillips, H. S., and Ferrara, N., 1993, Nature, 362:841-844.
15. Leon, S. P., Folkerth, R. D., and Black, P. M., 1996, Cancer, 77:362-372.
16. Takano, S., Yoshii, Y., Kondo, S., Suzuki, H., Maruno, T., Shirai, S., and Nose, T., 1996, Cancer Res, 56:2185-2190.
17. Leek, R. D., Lewis, C. E., Whitehouse, R., Greenall, M., Clarke, J., and Harris, A. L., 1996, Cancer Res, 56:4625-9.
18. Izycka-Swieszewska, E., Rzepko, R., Borowska-Lehman, J., Stempniewicz, M., and Sidorowicz, M., 2003, Folia Neuropathol, 41:15-21.
19. Bergers, G., Javaherian, K., Lo, K.-M., Folkman, J., and Hanahan, D., 1999, Science, 284:808-812.
20. Cheng, S. Y., Huang, H. J., Nagane, M., Ji, X. D., Wang, D., Shih, C. C., Arap, W., Huang, C. M., and Cavenee, W. K., 1996, Proc. Natl Acad Sci USA, 93:8502-8507.
21. Hsu, S. C., Volpert, O. V., Steck, P. A., Mikkelsen, T., Polverini, P. J., Rao, S., Chou, P., and Bouck, N. P., 1996, Cancer Res, 56:5684-5691.
22. Brooks, P. C., Stromblad, S., Klemke, R., Visscher, D., Sarkar, F. H., and Cheresh, D. A., 1995, J. Clin. Invest, 96:1815-1822.
23. Boehm, T., Folkman, J., Browder, T., and O'Reilly, M. S., 1997, Nature , 390:404-407.
24. Hanahan, D., and Folkman, J., 1996, Cell, 86:353-64.
25. Arap, W., Pasqualini, R., and Ruoslahti, E., 1998, Science, 279:377-380.
26. Friedlander, M., Brooks, P. C., Shaffer, R. W., Kincaid, C. M., Varner, J. A., and Cheresh, D. A., 1995, Science, 270:1500-2.
27. Berkman, R. A., Merrill, M. J., Reinhold, W. C., Monacci, W. T., Saxena, A., Clark, W. C., Robertson, J. T., Ali, I. U., and Oldfield, E. H., 1993, J Clin Invest, 91:153-159.

28. Samoto, K., Ikezaki, K., Ono, M., Shono, T., Kohno, K., Kuwano, M., and Fukui, M., 1995, Cancer Res, 55:1189-1193.
29. Obermair, A., Bancher Todesca, D., Bilgi, S., Kaider, A., Kohlberger, P., Mullauer Ertl, S., Leodolter, S., and Gitsch, G., 1997, J Natl Cancer Inst, 89:1212-1217.
30. Chaudhry, I. H., O'Donovan, D. G., Brenchley, P. E., Reid, H., and Roberts, I. S., 2001, Histopathology, 39:409-15.
31. Pintucci, G., Bikfalvi, A., Klein, S., and Rifkin, D. B., 1996, Semin Thromb Hemost, 22:517-524.
32. Jensen, R. L., 1998, Surg Neurol, 49:189-95, discussion 196.
33. Hirano, H., Lopes, M. B., Laws, E. R., Jr., Asakura, T., Goto, M., Carpenter, J. E., Karns, L. R., and VandenBerg, S. R., 1999, Neuro-oncol, 1:109-19.
34. Yu, H., and Rohan, T., 2000, J Natl Cancer Inst, 92:1472-89.
35. Trojan, L. A., Kopinski, P., Wei, M. X., Ly, A., Glogowska, A., Czarny, J., Shevelev, A., Przewlocki, R., Henin, D., and Trojan, J., 2002, Acta Biochim Pol, 49:979-90.
36. Mukherjee, P., Sotnikov, A. V., Mangian, H. J., Zhou, J. R., Visek, W. J. and Clinton, S. K., 1999, J Natl Cancer Inst, 91:512-523.
37. Kari, F. W., Dunn, S. E., French, J. E., and Barrett, J. C., 1999, J Nutr Health Aging, 3:92-101.
38. Lund, E. L., Spang Thomsen, M., Skovgaard Poulsen, H., and Kristjansen, P. E., 1998, Acta Neurol Scand, 97:52-62.
39. Bingle, L., Brown, N. J., and Lewis, C. E., 2002, J Pathol, 196:254-65.
40. Berse, B., Brown, L. F., Van de Water, L., Dvorak, H. F., and Senger, D. R., 1992, Mol Biol Cell, 3: 211-220.
41. Senger, D. R., Van de Water, L., Brown, L. F., Nagy, J. A., Yeo, K. T., Yeo, T. K., Berse, B., Jackman, R. W., Dvorak, A. M., and Dvorak, H. F., 1993, Cancer Metastasis Rev, 12:303-324.
42. Mukherjee, P., El-Abbadi, M.M., Kasperzyk, J. L., Ranes, M. K., and Seyfried, T. N., 2002, Br J Cancer, 86:1615-21.
43. Tannenbaum, A., 1959, In Physiopathology of Cancer,. Homburger, F., ed, 517-562. Paul B. Hober, NY.
44. Tannenbaum, A., 1942, Can Res, 2:460-467.
45. Weindruch, R., and Walford, R. L., 1988, The retardation of aging and disease by dietary restriction. Thomas, Springfield, IL.
46. Kritchevsky, D., 1999, Toxicol Sci, 52:13-6.
47. Greene, A. E., Todorova, M. T., and Seyfried, T. N., 2003, J Neurochem, 86:529-37.
48. Rous, P., 1914, J Exp Med 20:433-451.
49. Seyfried, T. N., Sanderson, T. M., El-Abbadi, M. M., McGowan, R., and Mukherjee, P., 2003, Br J Cancer, 89:1375-82.
50. Keenan, K. P., Ballam, G. C., Soper, K. A., Laroque, P., Coleman, J. B., and Dixit, R., 1999, Toxicol Sci 52:24-34.
51. Seyfried, T. N., El-Abbadi, M., and Roy, M. L., 1992, Mol Chem Neuropathol, 17:147-167.
52. Zimmerman, H. M., and Arnold, H., 1941, Cancer Res, 1:919-938.
53. Seyfried, T. N., Yu, R. K., Saito, M., and Albert, M., 1987, Cancer Res, 47:3538-3542.
54. Ranes, M. K., El-Abbadi, M., Manfredi, M. G., Mukherjee, P., Platt, F. M., and Seyfried, T. N., 2001, Br J Cancer, 84:1107-14.
55. Beecken, W. D., Fernandez, A., Joussen, A. M., Achilles, E. G., Flynn, E., Lo, K. M., Gillies, S. D., Javaherian, K., Folkman, J., and Shing, Y., 2001, J Natl Cancer Inst, 93:382-7.
56. Holmgren, L., O'Reilly, M. S., and Folkman, J., 1995, Nat Med, 1:149-153.
57. O'Reilly, M. S., Holmgren, L., Chen, C., and Folkman, J., 1996, Nat Med, 2:689-92.
58. Tanaka, T., Manome, Y., Wen, P., Kufe, D. W., and Fine, H. A., 1997, Nat Med, 3:437-42.
59. Mukherjee, P., Zhau, J.-R., Sotnikov, A. V., and Clinton, S. K., 1999, In Antiangiogenic Agents in Cancer Therapy, Teicher, B. A., ed, 237-261. Humana Press, Totowa, NJ.
60. Kerbel, R. S., Viloria-Petit, A., Klement, G., and Rak, J., 2000, Eur J Cancer, 36:1248-57.
61. Greene, A. E., Todorova, M. T., McGowan, R., and Seyfried, T. N., 2001, Epilepsia, 42:1371-8.
62. Clarke, D. D., and Sokoloff, L., 1999, In Basic Neurochemistry, Siegel, G. J., Agranoff, B. W., Albers, R. W., Fisher, S. K., and Uhler, M. D., eds, 637-669. Lippincott-Raven, New York.
63. Owen, O. E., Morgan, A. P., Kemp, H. G., Sullivan, J. M., Herrera, M. G., and Cahill, G. F., Jr., 1967, J Clin Invest, 46:1589-95.
64. Oudard, S., Boitier, E., Miccoli, L., Rousset, S., Dutrillaux, B., and Poupon, M. F., 1997, Anticancer Res, 17:1903-11.
65. Mies, G., Paschen, W., Ebhardt, G., and Hossmann, K. A., 1990, J Neurooncol, 9:17-28.
66. Aronen, H. J., Pardo, F. S., Kennedy, D. N., Belliveau, J. W., Packard, S. D., Hsu, D. W., Hochberg, F. H., Fischman, A. J., and Rosen, B. R., 2000, Clin Cancer Res, 6:2189-200.
67. Fearon, K. C., Borland, W., Preston, T., Tisdale, M. J., Shenkin, A., and Calman, K. C. 1988, Am J Clin Nutr, 47:42-8.
68. Roslin, M., Henriksson, R., Bergstrom, P., Ungerstedt, U., and Bergenheim, A. T., 2003, J Neurooncol, 61:151-60.
69. Lichtor, T., and Dohrmann, G. J., 1986, Neurosurgery, 19:896-9.
70. John, A. P., 2001, Med Hypotheses, 57:429-31.
71. Warberg, O., 1956, Science, 123:309-314.

72. Tisdale, M. J., and Brennan, R. A., 1983, Br J Cancer, 47:293-7.
73. Fredericks, M., and Ramsey, R. B., 1978, J Neurochem, 31:1529-31.
74. Nebeling, L. C., Miraldi, F., Shurin, S. B., and Lerner, E., 1995, J Am Coll Nutr, 14:202-8.
75. Todorova, M. T., Tandon, P., Madore, R. A., Stafstrom, C. E., and Seyfried, T. N., 2000, Epilepsia, 41:933-40.
76. Freeman, J. M., Freeman, J. B., and Kelly, M. T., 2000, The Ketogenic Diet: A Treatment for Epilepsy Demos, New York.
77. Nebeling, L. C., and Lerner, E., 1995, J Am Diet Assoc, 95:693-7.
78. Fearon, K. C., Tisdale, M. J., Preston, T., Plumb, J. A., and Calman, K. C., 1985, Br J Cancer, 52:87-92.
79. Bosland, M. C., Oakley-Girvan, I., and Whittemore, A. S., 1999, J Natl Cancer Inst, 91:489-91.
80. Kritchevsky, D., 1999, In Nutritional Oncology, Heber, D., Blackburn, G. L., and Go, V. L. W., eds. 5-10, Academic Press, Boston.
81. Wang, L., Yang, H., and Adamo, M. L., 2000, Biochem Biophys Res Commun, 269:336-46.
82. Straus, D. S., and Burke, E. J., 1995, Endocrinology, 136:365-8.
83. Strohman, R., 2002, Science, 296:701-3.
84. Veech, R. L., Chance, B., Kashiwaya, Y., Lardy, H. A., and Cahill, G. F., Jr., 2001, IUBMB Life, 51:241-7.
85. Greenspan, R. J., 2001, Nat Rev Genet, 2:383-7.
86. Aft, R. L., Zhang, F. W., and Gius, D., 2002, Br J Cancer, 87:805-12.
87. Lee, Y. J., Galoforo, S. S., Berns, C. M., Tong, W. P., Kim, H. R., and Corry, P. M., 1997, J Cell Sci, 110:Pt 5,681-6.
88. Shim, H., Chun, Y. S., Lewis, B. C., and Dang, C. V., 1998, Proc Natl Acad Sci USA, 95:1511-6.
89. Spindler, S. R., 2001, Ann N Y Acad Sci, 928:296-304.
90. Sharman, M. J., Kraemer, W. J., Love, D. M., Avery, N. G., Gomez, A. L., Scheett, T. P., and Volek, J. S., 2002, J Nutr, 132:1879-85.
91. Marcondes, S., Turko, I. V., and Murad, F., 2001, Proc Natl Acad Sci USA, 98:7146-51.
92. Kirsch, J. R., and D'Alecy, L. G., 1984, Stroke, 15:319-23.
93. Yu, B. P., Lee, D. W., Marler, C. G., and Choi, J. H., 1990, Proc Soc Exp Biol Med, 193:13-5.
94. Dong, W., Selgrade, M. K., Gilmour, I. M., Lange, R. W., Park, P., Luster, M. I., and Kari, F. W., 1998, Am J Respir Cell Mol Biol, 19:462-9.
95. Polverini, P. J., 1999, In Antiangiogenic Agents in Cancer Therapy, Teicher, B. A., ed, 65-75. Humana Press, Totowa, New Jersey.
96. Hirano, H., Tanioka, K., Yokoyama, S., Akiyama, S., and Kuratsu, J., 2001, J Neurosurg, 95:89-95.
97. Mitchell, R. A., and Bucala, R., 2000, Semin Cancer Biol, 10:359-66.
98. Badie, B., and Schartner, J. M., 2000, Neurosurgery, 46:957-61.
99. Sharkey, A. M., Charnock Jones, D. S., Boocock, C. A., Brown, K. D., and Smith, S. K., 1993, J Reprod Fertil, 99:609-6015.
100. Roeder, L. M., Poduslo, S. E., and Tildon, J. T., 1982, J Neurosci Res, 8:671-82.
101. Kleiber, M., 1975, The Fire of Life: An Introduction to Animal Energetics. Robert E. Krieger Publishing Co., Malabar, Florida.
102. Savendahl, L., and Underwood, L. E., 1999, J Nutr, 129:2005-8.
103. Shelton, H. M., 1974, Fasting for Renewal of Life. Amer Nat Hygene Soc, Inc., Tampa, FL.
104. Birt, D. F., Yaktine, A., and Duysen, E., 1999, J Nutr, 129:571S-574S.
105. Kadekaro, M., Ito, M., and Gross, P. M., 1988, Neuroendocrinology, 47:329-34.
106. Badruddoja, M. A., Krouwer, H. G., Rand, S. D., Rebro, K. J., Pathak, A. P., and Schmainda, K. M., 2003, Neuro-oncol, 5:235-43.
107. Goodman, L. S., and Gilman, A., 1975, The Pharmacological Basis of Therapeutics. Macmillan, Inc., New York.
108. Tisdale, M. J., 2001, Nutrition, 17:438-42.
109. Mukherjee, P., Abate, L. E., and Seyfried T. N., 2004, Clin Can Res, 10(16): 5322-29.