Why Radiation Oncology?

- Cancer associated metabolic changes may also reveal the importance of **protection against reactive oxygen species** (Coller et al. *The American Journal of Pathology*, Vol. 184, No. 1, January 2014)

- Radiation works by increasing the reactive oxygen species such as hydroxyl radical which causes DNA damage, but most importantly it **directly opposes the result of metabolic changes seen in cancer by creating more oxidative stress**.

Cell

Leading Edge
Review

Hallmarks of Cancer: The Next Generation

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The hallmarks of cancer comprise six biological capabilities acquired during the multistep development of human tumors. The hallmarks constitute an organizing principle for rationalizing the complexities of neoplastic disease. They include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. Underlying these hallmarks are genome instability, which generates the genetic diversity that expedites their acquisition, and inflammation, which fosters multiple hallmark functions. Conceptual progress in the last decade has added two emerging hallmarks of potential generality to this list—reprogramming of energy metabolism and evading immune destruction. In addition to cancer cells, tumors exhibit another dimension of complexity: they contain a repertoire of recruited, ostensibly normal cells that contribute to the acquisition of hallmark traits by creating the "tumor microenvironment." Recognition of the widespread applicability of these concepts will increasingly affect the development of new means to treat human cancer.

I would like to first provide major clues why oxidative stress plays an important role explaining the abnormal cancer metabolism and how this clinically relevant to radiation oncology.

Evidence #1: Dr. Douglas Spitz's (University of Iowa) *JBC* article published in 2005 shows that cancer cells died of oxidative stress before they run out of ATP energy. This a land mark *in vitro* study showing that if you cut down glucose influx and provide oxidative stress (that can also be induced by radiation therapy), you can get a significant cytotoxicity to cancer cells.

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 280, No. 6, Issue of February 11, pp. 4254–4263, 2005
Printed in U.S.A.

Mitochondrial $O_2^{\cdot-}$ and H_2O_2 Mediate Glucose Deprivation-induced Cytotoxicity and Oxidative Stress in Human Cancer Cells*

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Published, JBC Papers in Press, November 23, 2004, DOI 10.1074/jbc.M411662200

Iman M. Ahmad‡, Nukhet Aykin-Burns‡, Julia E. Sim§, Susan A. Walsh‡, Ryuji Higashikubo§, Garry R. Buettner‡, Sujatha Venkataraman‡, Michael A. Mackey¶‖, Shawn W. Flanagan‡, Larry W. Oberley‡, and Douglas R. Spitz‡§**

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The figures and table below demonstrate that when glucose metabolic flux is blocked combined radiation therapy can be extremely potent weapon to kill cancer cells.

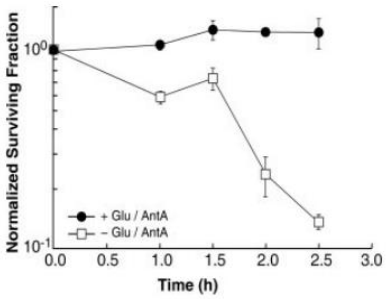

FIG. 2. **AntA induced cytotoxicity in a time dependent manner during glucose deprivation in GM00637G SV40-transformed cells.** Cells in the presence of 10 μM AntA were analyzed for clonogenic cell survival at various time intervals in the presence and absence of glucose. Errors represent ±1 S.D. of at least three cloning dishes counted for each point and taken from one treatment dish. Data were normalized to the time 0 h + glucose group.

TABLE I
ATP levels in GM00637G cells treated with +/−Glu for 2 or 8 h (n = 3)

Group	Glucose	ATP levels	
		2 h	8 h
		nmol/mg protein	
Control	+	0.253 ± 0.015	0.249 ± 0.037
	–	0.249 ± 0.024	0.270 ± 0.001
Ant A (10 μM)	+	0.260 ± 0.026	0.306 ± 0.040
	–	0.269 ± 0.008	0.263 ± 0.023
DNP (2 μM)	+	0.273 ± 0.046	0.258 ± 0.031
	–	0.251 ± 0.025	0.231 ± 0.031

The above figure and table are from Dr. Spitz's (University of Iowa) paper published in from *the Journal of Biological Chemistry* Vol. 280 No. 6, pp 4254 – 4263 published in 2005 showing that with oxidative stress induced by Antimycin A when glucose is depleted, cancer cells died of the oxidative stress not due to the ATP depletion, which is currently the mainstream view by the cancer metabolism research community. However, more convincing data is continuing to come out supporting the oxidative stress theory to explain the main drive for abnormal cancer metabolism.

Evidence #2: Animal study data supporting when ketogenic diet is given combined with radiation, you get a significant improvement in prognosis in malignant glioma mice.

OPEN ACCESS Freely available online PLoS one

The Ketogenic Diet Is an Effective Adjuvant to Radiation Therapy for the Treatment of Malignant Glioma

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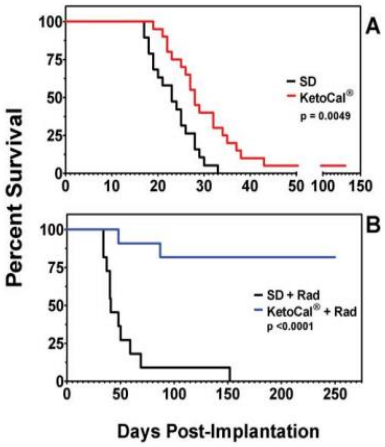
Abstract

Introduction: The ketogenic diet (KD) is a high-fat, low-carbohydrate diet that alters metabolism by increasing the level of ketone bodies in the blood. KetoCal® (KC) is a nutritionally complete, commercially available 4:1 (fat: carbohydrate+protein) ketogenic formula that is an effective non-pharmacologic treatment for the management of refractory pediatric epilepsy. Diet-induced ketosis causes changes to brain homeostasis that have potential for the treatment of other neurological diseases such as malignant gliomas.

Methods: We used an intracranial bioluminescent mouse model of malignant glioma. Following implantation animals were maintained on standard diet (SD) or KC. The mice received 2×4 Gy of whole brain radiation and tumor growth was followed by *in vivo* imaging.

Results: Animals fed KC had elevated levels of β-hydroxybutyrate ($p = 0.0173$) and an increased median survival of approximately 5 days relative to animals maintained on SD. KC plus radiation treatment were more than additive, and in 9 of 11 irradiated animals maintained on KC the bioluminescent signal from the tumor cells diminished below the level of detection ($p<0.0001$). Animals were switched to SD 101 days after implantation and no signs of tumor recurrence were seen for over 200 days.

Conclusions: KC significantly enhances the anti-tumor effect of radiation. This suggests that cellular metabolic alterations induced through KC may be useful as an adjuvant to the current standard of care for the treatment of human malignant gliomas.



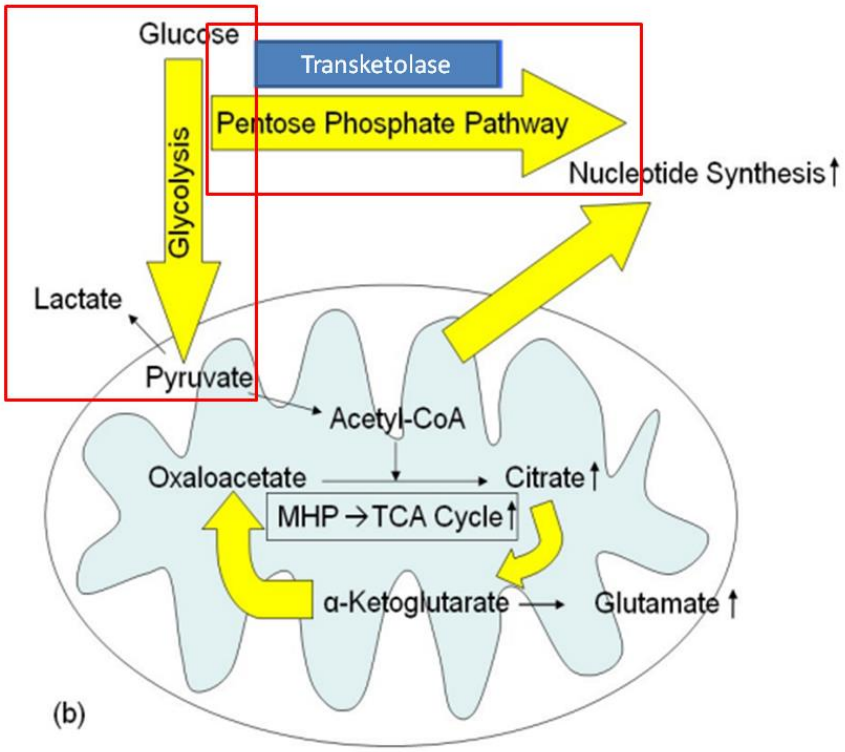
C

Treatment	Cohort Size	Median Survival (Days)
SD	19	23
KetoCal®	19	28
SD + Rad	11	41
KetoCal® + Rad	11	Undefined

Figure 1. KetoCal® enhances survival of glioma-bearing mice. Kaplan-Meier plot of survival in KC versus SD (A), radiation versus KC plus radiation (B). Animals on KC survived significantly longer when treated with KC alone ($p<0.005$), or when combined with radiation ($p<0.0001$). Results are a combination of (A) 4 separate experiments and (B) 2 separate experiments.
doi:10.1371/journal.pone.0036197.g001

The above figure is from *PLOS One* published in 2012:
http://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0036197&type=printable
This was an animal data produced by Dr. Adriana Scheck at Barrow Institute located in Phoenix, Arizona. She is currently conducting a human clinical trial to apply both ketogenic diet and radiation therapy for glioblastoma patients.

Evidence #3: The conventional Warburg effect was limited to the anaerobic glycolytic pathway, however, during the past decade, the up-regulation of the pentose phosphate pathway has been shown to be the expansion of the Warburg effect. Why? Again, **the oxidative stress**!



This article in *PNAS* was published recently corroborating the oxidative stress theory to explain abnormal cancer metabolism.

Transketolase counteracts oxidative stress to drive cancer development

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Edited by Tak W. Mak, The Campbell Family Institute for Breast Cancer Research at Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada, and approved December 24, 2015 (received for review May 5, 2015)

Cancer cells experience an increase in oxidative stress. The pentose phosphate pathway (PPP) is a major biochemical pathway that generates antioxidant NADPH. Here, we show that transketolase (TKT), an enzyme in the PPP, is required for cancer growth because of its ability to affect the production of NAPDH to counteract oxidative stress. We show that TKT expression is tightly regulated by the Nuclear Factor, Erythroid 2-Like 2 (NRF2)/Kelch-Like ECH-Associated Protein 1 (KEAP1)/BTB and CNC Homolog 1 (BACH1) oxidative stress sensor pathway in cancers. Disturbing the redox homeostasis of cancer cells by genetic knockdown or pharmacologic inhibition of TKT sensitizes cancer cells to existing targeted therapy (Sorafenib). Our study strengthens the notion that antioxidants are beneficial to cancer growth and highlights the therapeutic benefits of targeting pathways that generate antioxidants.

TKT | HCC | ROS | metabolism | PPP

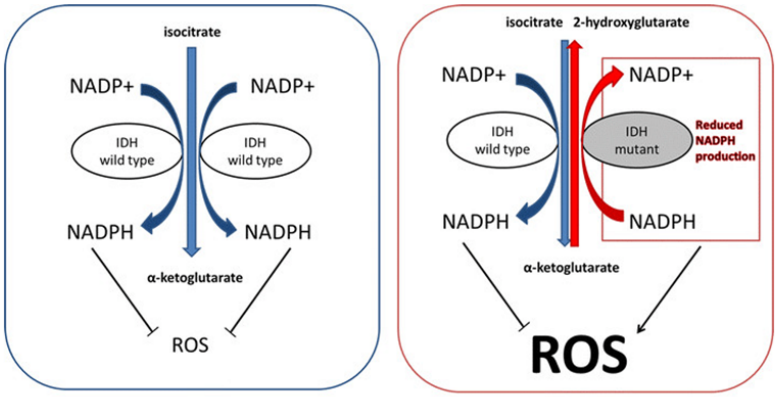
When cells are in need of nucleotides, the PPP produces ribose through the oxidative arm from G6P and the nonoxidative arm from F6P and G3P.

Although the PPP and glycolysis are equally important in the metabolism of cells, attention has been mostly drawn to the mechanisms by which glycolysis benefits tumor growth. Knowledge regarding the roles of the PPP in cancer cells is relatively scarce. Among all of the enzymes in the PPP, only the roles of G6PD and TKTL1 were briefly revealed in cancer. G6PD and TKTL1 were reported to be activated or overexpressed in cervical, lung, gastric, colorectal, and endometrial cancers (2–7). Suppressing TKTL1 in colorectal tumor cells reduced glucose uptake and lactate accumulation and enhanced sensitivity to oxidative stress (8).

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. It is the fifth most common cancer and the second leading cause of cancer deaths. The 5-y survival rate of HCC patients is less than 10% (9). Its high mortality rate is at

Evidence #4:

IDH Mutation is associated with better prognosis in glioblastoma: OXIDATIVE STRESS!



Peters *et al*. Amino Acids (2017) 49: 21-32

So will over expression of p53 kill cancer cells by increasing oxidative stress???

Free Radic Biol Med. 2008 March 1; 44(5): 826–834.

2-Deoxyglucose combined with wild type p53 over expression enhances cytotoxicity in human prostate cancer cells via oxidative stress

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Abstract

Over expression of the tumor suppressor gene, wild type p53 (wtp53), using adenoviral vectors (Adp53) has been suggested to kill cancer cells by hydroperoxide-mediated oxidative stress [1,2] and nutrient distress induced by the glucose analog, 2-deoxyglucose (2DG), has been suggested to enhance tumor cell killing by agents that induce oxidative stress via disrupting hydroperoxide metabolism [3,4]. In the current study clonogenic cell killing of PC-3 and DU-145 human prostate cancer cells (lacking functional p53) mediated by 4 h exposure to 50 plaque forming units (pfus)/ cell of Adp53 (that caused the enforced over expression of wtp53) was significantly enhanced by treatment with 2DG. Accumulation of glutathione disulfide was found to be significantly greater in both cell lines treated with 2DG+Adp53 and both cell lines treated with 2DG+Adp53 showed a ~2-fold increases in dihydroethidine (DHE) and 5-(and-6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate ($CDCFH_2$) oxidation, indicative of increased steady-state levels of $O_2^{\bullet-}$ and hydroperoxides, respectively. Finally, over expression of catalase or glutathione peroxidase using adenoviral vectors partially, but significantly, protected DU-145 cells from the toxicity induced by 2DG+Adp53 treatment. These results show that treatment of human prostate cancer cells with the combination of 2DG (a nutrient stress) and over expression of the tumor suppressor gene, wtp53, enhances clonogenic cell killing by a mechanism that involves oxidative stress as well as allowing for the speculation that inhibitors of glucose and hydroperoxide metabolism can be used in

Oxidative Stress is one of the main driving forces to explain the abnormal cancer metabolism.

- So cancer cells are not necessarily using abnormal glucose metabolism to supply energy. The reason is probably much more complex.
- At least, the study (Spitz's 2005 JBC article) demonstrated the abnormal glucose metabolism seems to be important way to protect cancer cells from the oxidative stress.
- The implication of this is very important for radiation oncology community since radiation generates "oxidative stress" to kill cancer cells.

Recently, The New York Times Magazine wrote this article above reflecting a huge interest of public to use dietary/metabolic approach to kill cancer quoting Dr. Lewis Cantley from Cornell University and Dr. Craig Thompson from MSKCC.

I believe radiation oncology is the ideal specialty to take advantage of this opportunity and I believe I am the ideal candidate who can make a contribution to better the way we treat cancer patients with radiation. Most importantly, this new treatment modality will enable patients to be part of the treatment and give more empowerment.