Reviewer's report

Title: Growth of human gastric cancer cells in nude mice is delayed by a ketogenic diet supplemented with omega-3 fatty acids and medium-chain triglycerides

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Reviewer: Thomas Seyfried

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General
The authors present an interesting study showing that a ketogenic diet supplemented with omega 3 fatty acids can increase survival in nude mice implanted with cells from a malignant human adenocarcinoma. It also appears that the diet therapy reduces tumor vascularity, which may account in part for the increased survival. There are a number of issues that the authors should consider to improve the accuracy of their study and the conclusions drawn.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. The authors showed that glucose metabolism was greater in the carcinoma cells than in the control PMBC control cells. It would be interesting to determine if the lactate production in the cultured tumor cells changes if beta-hydroxybutyrate is added to the culture medium. They mention preliminary results on this in discussion). These data should be shown. Tumor cell survival for at least 72 hrs in a low glucose/high ketone culture medium would suggest normal respiration in these cells. Such findings would also indicate that these cells do not show the Warburg effect. Warburg clearly specified that tumor cells have defective respiration. Survival in a low glucose/ketone-rich growth environment would refute the Warburg hypothesis for these tumor cells.

2. The authors mention that reduced body weight was not a factor in the smaller size of the tumors in the KD group compared to the SD group. However, it is interesting that body weights were smaller in the KD group than in the SD group over the first 20 days of the experiment, i.e., during the time when the diet had the greatest effect on tumor growth. The authors should conduct a linear regression analysis (using body weight as the independent variable and tumor volume as the dependent variable) over the first 20 days of the experiment to determine the relationship of body weight to tumor growth. The regression should also be conducted after the first 20 days. It is unusual to find a cancer therapy that extends mouse survival, which does not also reduce tumor weight and volume. Further comment is required to address this apparent discrepancy.

3. The authors show that the KD increased ketosis, but did not reduce glucose
levels. In this regard, the authors should reference the previous work of Fearon et al., Brit. J. Cancer 52:87-92, 1985; Amer J. Clinical Nut., 47:42-49, 1988. These investigators considered that the inability of their KD to reduce tumor growth was due to persistently high glucose levels. The authors of the present study should consider these previous observations. Also, glucose levels were reduced in the patients from the Nebeling study. What would the authors expect with regard to mouse survival if glucose levels were reduced in their study? The authors should also recognize that insulin resistance and gluconeogenesis could account in part for the elevated glucose levels under ketogenic diet feeding. The authors will need to measure serum insulin levels to support their claim that reduced insulin levels contribute to the anti-tumor effect of the unrestricted KD.

4. The glycolytic behavior of tumors persists even in the presence of oxygen. This is due to defective respiration according to Warburg’s extensive data on tumors. The authors mention on page 19 that oxygen and ketone bodies might sustain tumor cells in viable tumor zones. This possibility would refute Warburg’s hypothesis. Ketone bodies can only be metabolized for energy with near normal mitochondria and respiration. The authors need to carefully consider about their statements or present data to support their claims. The ability to reactivate respiration in tumor cells has not been clearly demonstrated in any previous study despite claims to the contrary. It would therefore be important to show that survival for 3-4 days is similar in the 23132/87 tumor cells grown in either high glucose medium or in low glucose medium (less than 0.02 mmol) supplemented with high ketone bodies (5-10 mmol). The switch from glucose to ketone bodies for energy should also be accompanied by reduced production of lactic acid. Such observations would provide strong evidence that the Warburg effect is not expressed in these tumor cells. The authors should present these data if they are available.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Data presentation can be improved by using means with either SD, SEM, or 95% CI. Data in Figure 6, 7, and 11 are confusing and could be better summarized rather than presenting data points for each mouse.

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being
published

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.