Reviewer's report

Title: Selenized milk casein in the diet of BALB/c nude mice reduces growth of intramammary MCF-7 tumors.

Version: 1 Date: 25 July 2013

Reviewer: Gabriele Ludewig

Reviewer's report:

Warrington and coworkers investigated the effects of different levels of selenized milk protein in the diet on the growth of implanted human mammary tumors in immunodeficient BALB/c nude mice. The authors describe the production of high-Se casein from cows’ milk and report that moderate and high selenium milk casein (up to 1.15 ppm) in defined rodent diets decreased the mean tumor volume compared to low levels of 0.16 ppm of selenium over the 10 weeks experimental period. A decrease in mean predicted tumor volume and of the number of large tumors was seen. High Se diets decreased the proportion of tumors with a maximum growth rate and increased the number of apoptotic cells in these tumors, even though no change in Bcl-2, Bax, or cyclinD1 was seen. The authors suggest that the turnover of cells, but not their nutrient supply is affected by the dairy Se.

MINOR ESSENTIAL REVISIONS:

GENERAL COMMENTS

These are interesting observations which support other reports in the literature about anti-cancer effects of selenium. However, several aspects need clarification. Particularly the modeling (data presented in Table 2) is very difficult to follow, seems to include a lot of assumptions which values should be excluded from the model, adds confusion by using the same abbreviations as for the measured data, and is in the end primarily used to justify a statement “Se-casein appears to affect cancer cell turnover but not supply or extraction of nutrients by the tumor”, which seems to be an over-interpretation of the existing, very variable data.

SPECIFIC COMMENTS

Abstract

1. Please mention in “Methods” that apoptotic cells and Bcl-2, Bax, and Cyclin D1 protein levels in tumors were determined.

2. In “Results” it should be mentioned that final tumor mass, BCL-2, Bax, and Cyclin D1 protein levels in tumors were not significantly affected by different Se levels in the diet.

3. The sentence “there was a significantly higher number of apoptotic cells in high-Se tumors as compared to low-Se tumors” should be moved from
Conclusions to Results.

Introduction

4. Selenium is an essential trace element, but also toxic at high concentration. Please indicate which dietary level is considered adequate (or too high or too low).

5. 2nd paragraph, 1st and 2nd sentence: both statements need a citation

6. p.5 first paragraph: “each increment in Se intake between 0.16 and 1.15 ppm of dry matter caused a decrease in tumor volume.” This statement suggests a strong concentration dependency which seems to be an overstatement considering the large variability in tumor size and growth pattern and the very small effect of the 0.51ppm Se diet.

Materials and Methods

7. p.5 Casein isolation paragraph, 1st sentence: please specify what you mean by “basal diet”.

8. p.6, Casein isolation and diet formulation: “AIN-76 diets (Research Diets Inc)” is confusing. I assume you mean the purified rodent diet AIN-76A, and that you prepared it yourself, since you had to add different caseins, or did the company prepare it with the casein provided from you? Please explain why you chose these dietary Se levels!

9. p.6 Animal trials: please indicate whether or not food and water was provided at libitum. Please indicate whether you measured food consumption. Did you determine the body weight during the experiment and at the end? How many animals did you have in each dietary group at the start of the experiment and did any animals die during the experiment.

10. p.6 Animal trials cont.: “Once tumor volume reached …”. This sentence raises some concern about differences in tumor growth as strong modulating factor in the experiment. Please add information about the time period needed from implantation to inclusion in the dietary study for the animals. Although you randomly assigned the animals to dietary groups, did you follow the development of tumor growth in individual animals and analyzed whether there is a correlation between tumor growth before and after start on the diets?

11. Statistical Analysis paragraph: did you try to compare the lowest Se diet group (Low adequate Se level) individually with the other groups? You could indicate significance between this group and others with an asterisk in the figures in addition of giving the p value for the linear trend.

12. p.9 Statistical Analysis cont: You list several parameters that resulted in an exclusion of animals from the data set, “mice put on treatment 3 weeks after inoculation or later”, “tumors with a grad 0”, “if their final volume was below 30 mm2”. Were these animals/tumors only not considered for the data in Table 2 or for all figures as well?

13. p.10 Statistical Analysis cont: “Because of the large variation around mean tumor growth characteristics within treatment”. You could add “(see Figure 1)” to
examine what you mean with this statement.

Results

14. p.10 Tumor growth, line 1f: “exhibited exponential growth to a plateau (Figure 1)”. I have a hard time to see a plateau for most of the tumors in Fig.1. Please explain better.

15. p.10 Tumor growth, line 3f: “a tendency for final tumor mass to decrease (p=0.09; Fig 2b).” I see a downward trend (decrease) in volume for some tumors in Fig 1. Figure 2 shows a non-significant lower final tumor mass with the two higher Se diets.

16. p.11 line 3: “one mouse on 0.51ppm Se was excluded”. In addition the Vmax in this group is very high (Table 2). Please address this.

17. p.11 Tissue analysis line 3: “the DNA nick-end labeling also increased 2.4-fold with increasing Se level (Fig 6e).” This statement suggests a strict linear increase with concentration, but the 0.85ppm Se concentration is probably not significantly different from the 0.16ppm level.

Discussion

18. p.12 line 1: “Our findings show, for the first time, that dietary Se is effective in reducing growth of human mammary tumors in vivo.” This statement seems to be incorrect, since Chen and coworkers just published a similar study (Chen et al., 2013). The novelty of this study is the use of “casein isolated from milk of cows fed Se-yeast in their diet.”

19. p.12 line 5: “Number of large tumors after 8 weeks” please add “(tumors with $V_{final} > 500 \text{ mm}^3$)”

20. p.12 line 7-9: “Although … growth rate was reduced, the maximum volume that tumors were predicted to reach ($V_{max}$)…” This is very confusing and needs to be clarified. Please add that you are here talking about the Gompertz fits, the estimated $V_{max}$ which was modeled using the growth data. It may help to use other abbreviations for the modeled data, like $V_{max-G}$ or something similar to distinguish this $V_{max}$ from the measured data.

21. p.13 end of page: “In vitro, the IC50 of SeMet against MCF-7 was 45 uM…we found in mammary tumors the equivalent of 23 uM SeMet…slightly less than the IC50.” It would be interesting to know the Se concentration in normal tissues? Please discuss why cancer cells are more vulnerable, i.e. is there any evidence that cancer cells accumulate SeMet more than normal cells or that cancer cell metabolize it differently, preferably to the postulated active form methylselenol (Zheng et al ..) or that they are more vulnerable due to specific changes?

22. p.14 end of 2nd paragraph: “…suggesting an alternative apoptotic pathway”. You may want to mention glutathione peroxidase 4, which is a Se-dependent enzyme and was shown to influence apoptosis (Brigelius-Flohe and Maiorino, 2013).

23. p.14 end of 3rd paragraph: “..cyclin D1-mediated cell cycle progression does not appear to be responsible for the inhibitory effects of Se-casein on tumor
growth.” You may want to mention another option here. A recent study showed an involvement of selenium-binding protein 1 in reduced proliferation of MCF-7 cells in the presence of MetSe (Zhang et al., 2013). Also, the alternative mechanisms mentioned, i.e. caspase activation and inactivation of Akt and ERK1/2, could be analyzed.

24. p.15 line 2: define SeMSC

25. p.15 first or second paragraph: please provide information about possible toxic effects at high Se levels, like type 2 diabetes (Rayman and Stranges, 2013), or of wrong levels or wrong form possibly enhancing tumor growth and metastasis (Cassidy et al., 2013; Chen et al., 2013).

26. p. 15 2nd paragraph: “Modeling tumor dynamics suggest that a diet high in Se-casein decreases the maximum growth rate.” The modeling data do not show linear concentration dependency and overall seem to be based on a lot of assumption. More convincing data to back up this statement is needed.

27. p.16 1st sentence: “This study showed Se-casein to be an effective treatment of breast cancer, suggesting its potential role in adjuvant therapy.” This is an overstatement considering the limited amount of data in this study and needs to be modified. The authors could add a sentence about further experiment that are needed to shine light on the mechanism for the observed effects and eliminate safety concerns.

28. Figure 1: use the same scale for all figures, add the Se level in the left upper corner and provide the number of tumors (n) in each treatment.

CITED LITERATURE

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable
**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests