Reviewer's report

Title: Dietary grape polyphenol resveratrol increases mammary tumor growth and metastasis in immunocompromised mice

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Reviewer: Niina Saarinen

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The work by Castillo-Pichardo et al. aims to describe the effects of different doses (0.5, 5, or 50 mg/kg BWT) of orally administered resveratrol on growth of established orthotopic human MDA-MB-231 and MDA-MB-435 breast cancer tumors. The authors have selected these two estrogen receptor alpha negative cell lines as representatives of cells with different metastatic potential. The both cell lines used are GFP transfected allowing imaging of the primary tumors in vivo and metastases primarily ex vivo.

Major Compulsatory Revisions

Comment 1. One million tumor cells were injected into mammary gland in matrigel and resveratrol/vehicle interventions started after 1 week. The study claim to investigate the resveratrol effects on established tumors. Within one week, however, all cancer cell injected mammary sites are not yet established to real solid palpable tumors. Some aggressively growing tumors may have developed. Thus, this study set up does not solely investigate resveratrol effects on established tumors but rather on tumor development post initiation.

Comment 2. The tumor growth curves in Figure 1 are confusing and require modifications/clarifications as described below:

I. It is not clear from the manuscript text or from the figure legends how many of the injected tumor sites developed into growing tumors, and how those sites that did not grow to palpable tumors or were regressing below the original signals were treated (were they excluded or included).

II. There were no statistically significant differences between the treatment groups indicating very large variation in the obtained fluorescence signals during the study. This raises a concern about the relevance of using average growth curves for presenting the data from very scattered/ non-parametric data distribution.

III. The average of whole body fluorescence is provided as an indicator of mammary fat pad tumors, even in mice with large metastatic masses. Were those metastatic masses visible in whole body scans and how those were corrected?

IV. At the end of the experiment, tumor samples were collected for further analysis and example figures of tumors is given in Figure 1B. However, the
relevance of Figure 1B is not clear. These are figures of excised tumors in different treatment groups but no data based on this kind of figures is presented. In figure 1C, day 108 data points are unnecessarily repeated. Instead of current Figure 1C, a bar figure the ex vivo tumor scan data could be provided along with the actual tumor sizes and/or weights. This actual tumor size data is missing completely from the manuscript and should be provided.

These points regarding data presented in Figure 1 should be re-considered and clarified.

Comment 3. Please provide data or a previous source publication where fluorescence signal (obtained with a scanning method used in this work) of mammary xenograft tumors originated from the cell lines used in this study are correlated with the actual tumor volumes in vivo.

Comment 4. In all figures, respective statistical methods used for comparisons should be provided.

Comment 5. Were body weights different between groups during experiments? Please, add this information.

Comment 6. The bioavailability of resveratrol is low. What was resveratrol serum/tumor tissue concentrations in the treatment groups?

Comment 7. Table 1. In its present form it is not clear if the percentage of mice with metastases include those mice that did not develop primary tumors to injected sites. Statistical analyses of the presented data are missing and should be provided.

Comment 8. In materials and methods section, bones excised at necropsy should be specified.

Comment 9. Based on the study set up and presented data, the conclusion “…our findings demonstrate a cancer promoting role for low dietary concentrations of resveratrol in breast cancer” is misleading and has to be changed. The presented data rather demonstrates no statistically significant difference in the growth of primary MDA-MB-231 or MDA-MB-435 tumors in mice orally gavaged with resveratrol. However, the data indicates increased Rac activity both in MDA-MB-231 and MDA-MB-435 tumors after the highest 50 mg/kg BW exposure to resveratrol. This indicates possibility for Rac mediated effects by resveratrol in the tumors although the downstream mediators showed no significant changes in their expression. The dose responses/ low dose resveratrol responses to metastatic potential and Rac activity are inconsistent. Therefore, the presented data does not clearly support the statements on low dose resveratrol effects. Therefore, the conclusive statements should be reconsidered and modified.

Minor Essential Revisions

Comment 10. Please, justify the use of different immonocompromized hosts (e.g. SCIDs and athymic nudes) for MDA-MB-231 and MDA-MB-345 mammary
tumors.

Comment 11. Abstract conclusion; In this study resveratrol was gavaged not administered in diet. Therefore this work does not support statements about dietary administration of resveratrol. Please, change text accordingly.

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.