Author's response to reviews

Title: Muscadine Grape Skin Extract Reverts Snail-Mediated Epithelial Mesenchymal Transition via Superoxide Species in Human Prostate Cancer Cells

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Version: 2
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Author's response to reviews: see over
Reviewer's report
Title: Muscadine Grape Skin Extract Inhibits Snail-Mediated Epithelial Mesenchymal Transition via Superoxide Species in Human Prostate Cancer Cells
Version: 1
Date: 24 February 2014
Reviewer: Violeta Colova-Tsolova

Reviewer's report:
Minor Essential Revisions:
1. In section Background Paragraph 1: "Muscadine Grape Skin Extract (MSKE) derived from the muscadine grape (Vitis rotundifolia) differs from the more common red grapes used to produce red wines" - needs editing.
   Based on the skin color muscadine varieties are referred respectively as bronze and purple vs white and red for all other grapes.

Response: We thank the Reviewer for the constructive criticisms and have addressed each point. On page 4 Lines 73-76 we slightly modified our sentence and added a sentence to reflect the reviewers’ comments along with an additional reference to give the difference between muscadine grapes and other grapes. We stated that:

Muscadine Grape Skin Extract (MSKE) derived from the muscadine grape (Vitis rotundifolia) differs from the more common red grapes used to produce red wines, which normally contain resveratrol. Based on the skin color, muscadine varieties are referred respectively as bronze and purple compared to white and red for all other grapes [2].

2. Paragraph 4: "The ARCaP prostate cancer model utilized by Zhau et al demonstrated ...." the reference needs to be completed

Response: Line 113 on page 5 of the Background information we completed the Zhau et al reference.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests: I declare that I have no competing interests' bellow.
Reviewer's report
Title: Muscadine Grape Skin Extract Inhibits Snail-Mediated Epithelial Mesenchymal Transition via Superoxide Species in Human Prostate Cancer Cells
Version: 1 Date: 9 February 2014
Reviewer: Cesar Nopo-Olazabal

Reviewer's report:
Major Essential Revisions
1. Review title to avoid confusion. The manuscript shows important evidence of a relationship between MSKE and inhibition of Snail-mediated EMT but probably more experimental work is needed to completely solve this question. This can be implied form the language used in the Conclusions sections when the authors make us of word such as "could" or "may". Example: "Muscadine grape extract may inhibit Snail-mediated epithelial mesenchymal transition via superoxide species in human prostate cancer cells".

Response: The comments presented by the reviewer are well received. Based on the data presented in figure 4, where we saw after treatment with 5 µg/ml MSKE, there was a reversion of Snail-mediated EMT as shown by decreased expression of Vimentin and Snail and increased expression of E-cadherin, we have changed the title to more accurately reflect our observations (See Page 1, Line 1 and 2) The new title is Muscadine Grape Skin Extract Reverts Snail-Mediated Epithelial Mesenchymal Transition via Superoxide Species in Human Prostate Cancer Cells

As such we have also modified our Conclusions to remove the words “can” or “could” and definitively state that MSKE antagonizes Snail expression and reverts EMT. The new statement reads as follows (Page 17, Lines 369-372):

Collectively, our results indicate that Snail leads to increased levels of mitochondrial superoxide and EMT. Furthermore, MSKE and SOD reverts EMT by targeting Snail expression, underscoring the importance of targeting these pathways with various inhibitors and antioxidants.

Minor Essential Revisions
1. Review keywords. Keywords are used to index and search information. The journal allows up to 10 keywords, make use of all of them if possible. Is MSKE a better keyword than the full name "Muscadine grape skin extract"?

Response: In the Keywords Page 3, Line 52: we changed MSKE to Muscadine Grape Skin Extract. We have also added “Reactive Oxygen Species”

2. Names that are abbreviated should be expanded the first time they are mentioned or cited followed by their abbreviation or included in the Abbreviations section. This has been done in most cases but for instance ROS is mentioned for the first time in the Abstract/Background section and not in the Abstract/Methods section. Other abbreviations without expanded names in the manuscript:
TGF-beta, MAPK ERK, MMP-3, JAK/STAT, HRP, G418, etc.

**Response:** We have made sure to elaborate on any abbreviations utilized in the article, especially the ones within the introduction that we missed before.

Page 2, Line 31: We changed expanded the ROS abbreviation to Reactive Oxygen Species
Page 2, Line 43: We changed expanded the SOD abbreviation to Superoxide Dismutase
Page 4, Line 88: We expanded the TGF-beta abbreviation
Page 4, Line 89: We expanded the MAPK/ERK abbreviation
Page 4, Line 91: We expanded the MMP-3 abbreviation
Page 5, Line 108: We expanded the JAK/STAT abbreviation
Page 7, Line 148: We expanded the G418 to Geneticin
Page 7, Line 154: We expanded the HRP abbreviation
Page 9, Line 205: We expanded the NAC abbreviation

3. Page 7, line 9: It says Milipore, it should be Millipore

**Response:** In the Methods section/Reagents, Line 149 changed Milipore to Millipore

4. Page 9, line 6: HEPES/PBS and HydroCy3 volumes should be included

**Response:** In the Methods section/In vitro measurement of Superoxide with HydroCy3…page 9 Line 190 and line 191…media with 90µL PBS/HEPES buffer plus 10µL of 25 µM Hydro-Cy3

5. Page 9, last line: "5 h later..." come after a period, it should be "Five h later..."

Response: In Methods/In vitro cell migration assay section page 10 Line 206 we changed “5 h later… To Five h later…

6. Page 10, line 1: Replace "was" by "were"

**Response:** In Methods/In vitro cell migration assay section.. page 10 Line 207 was changed to were

7. Page 12, line 1: It says "Levels", it should be "levels"

**Response:** In the Results/Snail increases Superoxide levels in LNCaP cells…page 12 Line 252 Levels was changed to levels

8. Page 16, line 14: It says "... an MET.", it should be "... a MET."

**Response:** Discussion section page 15 Line 355 was changed to…could significantly……by a MET

9. Review format references are listed in the journal.

**Response:** In the Reference section which starts on page 19, Lines 409 through 496 we re-formatted the reference for the journal
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.