Author’s response to reviews

Title: Matrix Association Region/Scaffold Attachment Region: The Crucial Player in Defining the Positions of Chromosome Breaks Mediated by Bile Acid-Induced Apoptosis in Nasopharyngeal Epithelial Cells

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Dr. Matteo Pasini
Editor
BMC Medical Genomics

Dear Dr. Matteo Pasini,

Submission of revised manuscript (MGNM-D-18-00120) entitled “Matrix Association Region/Scaffold Attachment Region: The Crucial Player in Defining the Position of Chromosome Breaks Mediated by Bile Acid-Induced Apoptosis in Nasopharyngeal Epithelial Cells”

First of all, we would like to thank the editor and reviewers for very constructive comments and suggestions. We would like to submit this revised manuscript after addressing the comments given by the reviewers.

Reviewer #1:
Title

The title is clear and represented the content.

Abstract

Background is not clear the idea and many gap information. For example, what correlation between BA and chromosomal cleavage, the two information should be arranged in the logic manner. This finding more related to prove correlation between BA with the chromosomal break rather than BA-induced apoptosis, since no apoptosis data in this experiment. Therefore, the abstract should be focus on the experiment data.

We agree with the comment given by reviewer #1. Thus, we adjusted the text in the section of ‘Abstract’. The amendments can be viewed in:

- We added a sentence to strengthen the correlation between BA and chromosomal cleavage (page 2, lines 5-6).
- We rephrased the statement of our hypothesis (page 2, lines 9-10).
- We omitted a sentence in subsection of ‘Conclusion’: ‘Our findings suggest a role for BA-induced apoptosis in mediating chromosome rearrangements in NPC.’ (initially in page 3, lines 5-6).
- We rephrased the statement of our conclusion (page 3, lines 7-8).

Introduction

Two scenarios were presented the most possible NPC, one is activation CAD mediated chromosomal cleavage, and second is inflammation process induce by BA. After comparison, authors assume link between the both theory by inducing ROS. However, the detail mechanism BA induce ROS was missed in the manuscript. Also, experimental data neglected ROS existence in the cell.

We have previously done measurement of ROS level in BA-treated nasopharyngeal epithelial cells. These results have been published in our previous report [BMC Cancer (2018) 18:409, Figure 4 and Figure 5]. We have stated these findings and cited this report (Reference [76]) in page 6, lines 19-21.

The detailed mechanism in which BA may induce ROS has been described in our previous report [BMC Cancer (2018) 18:409]. It is known that in BA-induced apoptosis, ROS is mainly generated through the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases [Ignacio et al. 2011; Yerushalmi et al., 2001]. In addition, due to the hydrophobic nature of BA, BA may directly diffuse into the cytosol resulting in mitochondrial perturbations.
This can lead to subsequent alteration in oxidative phosphorylation which causes excessive ROS formation [Ignacio et al., 2011]. This ROS generation is strongly associated with the onset of mitochondrial permeability transition (MPT) which is an important feature of BA-induced apoptosis [Yerushalmi et al., 2001].


Result

Visualization of data need to be re organized in to more compact, if the data shown in the figure, then should avoid the similar data in the table. Data visualization, not easy to be understood, especially data from bioinformatics.

We appreciate the comment from the reviewer. However, we feel that certain data are essential in both table form and figure form. The data presented in tables are the sequence composition of MRS, exact nucleotide positions and the length which could not be easily shown by figures. However, figures provide a summary and an overview of those data.

Discussion

Discussion bereaved focus to the aim of study, remove the general information will be better to improve manuscript more clear information for reader.

We have done extensive revision in the Discussion section. The amendments are as follows:

• Paragraph 2 which describes the concentration of BA used in this study had been placed in the Methods section (page 10, lines 16-19; page11, lines 1-4).

• Paragraph 3 which describes the acidic pH used in this study had been placed in the Methods section (page 11, lines 5-14).

Conclusion
Author concluded that BA-induced apoptosis caused chromosomal breakages in NPC shared similar with leukemia, that lead opinion causative agent of leukemia. I suggest rephrasing sentences more neutral based experimental data rather than based on assumption.

We would like to clarify that the conclusion that ‘the positions of these BA-induced chromosome breaks shared high similarity with those identified in patients with leukaemia’ was made based on the sequencing data obtained in the present study, not based on assumption. These findings have been described in the subsection of ‘Sequencing results’ (page 21, lines 5-11).

Reviewer #2

REVIEWER COMMENTS FROM REPORT: Nasopharyngeal carcinoma (NPC) is caused by gastric refluxes, which contain bile acid, a proponent of apoptosis and carcinoma driver. The authors hypothesise that bile acid induces chromosomal breaks at hotspot regions.

Using normal nasopharyngeal epithelial cells, the authors investigated one of these chromosomal breakage hotspot (AF9) and found that bile acid did induce chromosomal rearrangements at the AF9 loci, which is rearranged in ALL leukemia patients.

Although long, the Introduction is well written and informative.

The Bibliography seems to be outdated with very few recent research articles.

We have updated the Bibliography. A few recent publications have been included:


The Methods section is well detailed. However, the source of normal cell lines being other academics, how did the authors validate the authenticity of the cells? What was the passage number? For how many passages the cells kept in culture?

NP69 is an immortalised nasopharyngeal epithelial cell line which was established by transfection with SV40 large T oncogene. It is not a primary cell line. It retains some characteristics of normal nasopharyngeal epithelial cells and is non-tumourigenic. This cell line
may provide potential nasopharyngeal epithelial cell model for studying mechanisms involved in the tumourigenesis of NPC (Tsao et al., 2002).


Also, catalogue numbers for reagents could be useful.

The catalogue numbers for reagents have been included in the subsection of ‘Cell lines and chemicals’ (page 8, lines 10-19; page 9, lines 1-5).

The Authors have thoroughly investigated the effect of bile acid on the breakage of AF9 loci.

REQUESTED REVISIONS:

A few typos and corrections (highlighted in CAPITAL):

- Page 4, around line 25 in PDF file: The typical GORD symptoms such as heartburn and acid regurgitation may not BE present in half of these patients [20].

We have made the amendment accordingly, on page 4, line 12.

- Page 5, around lines 20-27: In addition, BA has also been shown to have the carcinogenic effect in human hypopharyngeal squamous carcinoma FaDu cells through epithelial-mesenchymal transition (EMT) [REF 60?]. EMT is a major pathway related to cancer invasion and metastasis [60 A RECENT REVIEW ON EMT AND CANCER SHOULD BE INCLUDED].

We have included a recent review on EMT and cancer (Page 5, line 13):


- Page 5, from line 34, there are many "MAY". It makes the NF-kappaB area seem very hypothetical while NF-kappaB is known to play a central role in inflammatory responses. A review (such as PMID 22257950) could be referenced.

We have rephrased the sentences (page 5, lines 17-18, 18-19, 21-22) and cited PMID 22257950 in the text (page 5, line 13).
- Page 14, line 45, "intron 4 (MAR/SARs 22-26 in Table 1) WERE found to contain five MAR/SAR sites" and line 53, "(MAR/SAR 29 in Table 1) WERE found to contain one MAR/SAR site"

We have made the amendments accordingly (page 15, lines 11-12, lines 13-15).

- Page 9, lines 46-58 are not necessary. Could mention something briefly about it in the Discussion if useful.

This paragraph is essential in the ‘Methods’ section because it explains the reason why we are setting 250 bp as the maximal distance between the 8 bp sequence element and the 16 bp sequence element. On page 10, line 2, we have included our recently published paper which described this in detail (Reference [91]).

- Page 10, lines 34-44. Although there is a reference, the amount (μM or mM) for each component should be specified.

We have stated the concentrations of the five bile salts in the subsection of ‘Preparations of BA cocktail and media for BA treatment’ (page 10, lines 18-19).

A suggestion to increase impact and broaden interest would be to perform next-generation sequencing (NGS) of bile acid-treated cells to identify breakage points genome-wide.

We would like to thank the reviewer for the very constructive comments. This suggestion will be considered in future experiments.

We would really appreciate if this manuscript could be considered for publication in BMC Medical Genomics. We look forward to your reply.

Thank you.

Yours sincerely

Sai-Peng Sim