Author's response to reviews

Title: Peptides specific for esophageal cancer cells were selected from a phage display peptide library

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Author's response to reviews: see over
Dear Editor,

Thank you for reviewing the manuscript we previously submitted. The comments and feedback we received were very helpful.

Accordingly, we have provided point-by-point responses to each of the reviewers’ concerns below, and changes in the manuscript are shown in red.

We hope that you will find the revised version of our manuscript to be suitable for publication by the Journal of Cardiothoracic Surgery.

Thank you in advance for your further time and consideration of our manuscript.

Sincerely,

Point-by-point responses

Reviewer's report:
This study screened a phage display peptide library for peptides specifically binding to esophageal cancer cells using a novel approach, phage display technique, and obtained two peptides that exhibited high binding affinity and specificity for esophageal cancer cells. As the authors stated, this study appears
to be the first attempt to use phage display to identify sequences that bind to
human esophageal cancer cells, and thus, the findings are of some clinical
significance.

Reply: Thank you for considering the clinical significance of our study.

Minor Essential Revisions

1. Abstract: A conclusive statement directly drawn from the major findings should
be added and the current sentence should be shortened.

Reply: Thank you for this feedback. We have now modified the Conclusions section of
the Abstract.

2. Where did the normal esophageal tissue sections come from? What were the
“normal esophageal cells” used as a control for Eca109 cells and where were
they from?

Reply: Primary esophageal cells were used, and additional details have been added to the
Materials and methods section.

3. Please explain what anti-M13 is and why you chose it for “validation” in ELISA
and fluorescence array. Please clarify if anti-M13 was the “primary antibody”
sued for IHC.

Reply: We have provided additional details regarding the anti-M13 used in the Methods
section.
4. Selection of 20 clones and definition of “clones specifically bound to Eca109 cells” need to be described in the Methods.

Reply: Thank you for this feedback. We have now added a description of the positive phage clones that were selected for further characterization in the Methods section.

5. The title seems too brief, and may be modified as “Screening of peptides specific for esophageal cancer cells from a phage display peptide library”.

Reply: Thank you for this feedback. We have modified the title as suggested.

6. Introduction: phage display technology should be described in more detail, and the first paragraph in the Discussion should be incorporated here.

Reply: Thank you for this feedback. We have made the changes indicated.

7. More descriptions should be provided in section “Selection of positive clones and amplification”.

Reply: Thank you for this feedback. We have provided additional details regarding the selection and amplification of positive phage clones in the Methods section.

8. Please describe or define “blue phage plaque” and “positive phage clone” clearly.

Reply: Thank you for this feedback. We now define these terms in the Methods section.
9. In the sentence “There was no homology observed between the two sequences (Table 2), or between other protein sequences of the Amersham Biosciences database”, what is meant by “between other protein sequences of the Amersham Biosciences database”? Do you mean “There was no homology between the two screened sequences and any protein sequences in the Amersham Biosciences”?  

Reply: We apologize for this confusion. We have now revised our statement to clarify the statement regarding homology.

10. Discussion should focus on the major findings of the present preliminary study. More importantly, how to further identify and characterize the two peptides, the clinical implications of the findings and limitations of the present study needs to be commented.  

Reply: Thank you for this feedback. We have now revised the Discussion to address these points, particularly the clinical implications and limitations of the present study.

11. The term “subtraction biopanning” used in Table 1 should be described/mentioned in the Methods.  

Reply: Thank you for this feedback. We have now specifically cited and revised the section of the Methods that refers to subtraction biopanning.

12. Overall, the manuscript was fairly prepared in a good organization; however, it can be further benefited from language proofreading by a native speaker.
Reply: Thank you for this feedback. We have had our revised manuscript reviewed by a senior medical editor.

**Level of interest:** An article of importance in its field

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

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

None