Author's response to reviews

Title: The Role of Cytochrome c Oxidase Subunit Va in Non-small Cell Lung Carcinoma Cells: Association with Migration, Invasion and Prediction of Distant Metastasis

Authors:

Wen-Liang Chen (wenurea@yahoo.com.tw)
Kuang-Tai Kuo (doc2738h@gmail.com)
Teh-Ying Chou (tychou@vghtpe.gov.tw)
Chien-Lung Chen (chainlong.tw@yahoo.com.tw)
Chih-Hao Wang (blinder988@yahoo.com.tw)
Yau-Huei Wei (joeman@ym.edu.tw)
Liang-Shun Wang (wangls72269@yahoo.com.tw)

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Author's response to reviews: see over
To the honorable editor,

I am returning you the re-revised form of the previously submitted manuscript for your review and approval. We have studied the reviewers’ comments and have revised the manuscript accordingly.

We added the reference 13 for the migration study in the method section. According to the reviewer’s suggestions, we replaced all of the data in the knockdown experiments. Previously, we used the CL1-5 cells as the representative to knockdown COX Va, and conducted corresponding migration, invasion, RT-PCR for COX Va and Bcl-2, as well as gelatin zymography. Now we replace them with a new set of data obtained from the H2009 cells. However, neither the results nor the conclusions are changed.

Finally, the authors deeply appreciate the editor for the opportunity to response the reviewers’ comments and to improve this article.

Liang-Shun Wang, MD, Professor
Division of Thoracic Surgery, Department of Surgery, Shuang Ho Hospital, Taipei Medical University & Graduate Institute of Clinical Medicine, Taipei Medical University
No.291, Zhongzheng Rd., Zhonghe District, New Taipei City 235, Taiwan
Phone: +886-2-22490088 ext. 8886; Fax: +886-2-22490088 ext. 8889
E-mail: wangls72269@yahoo.com.tw
To Reviewer 1:

Review’s 2nd reply

: The reference for those experimental methods should be added for clarification for improvement of this manuscript.

→ We thank the reviewer’s remind. We had added the reference 13 for this part.

To Reviewer 2:

Major Compulsory Revisions

The authors have addressed most of the points raised by this reviewer. However, since the authors present new data regarding Bcl-2 in Fig.7 several questions remain.

1) The mRNA expression of Bcl-2 is definitely the strongest in the H2009 cell line. Why did the authors choose the CL-1-5 cell line for the knockdown experiments in Fig. 7B? In this case, almost every researcher would use the cell line with its strongest expression. I can understand that the authors would like to present data for the same cell line (CL-1-5) they presented for the knockdown experiments for COX Va, but even here is the mRNA expression for COX Va stronger in the H2009 cell line. The work of the authors would benefit if they also could present experimental results for the H2009 cell line which they obtained for the CL-1-5 cell line in Fig. 5 and 7B.

→ We thank the reviewer’s comments. We had replaced the CL1-5 cells with H2009 cells as the representative and repeated all the corresponding experiments. The Fig.5, and 7B and even Fig.6C were changed accordingly.

2) From their experiments shown in Fig. 7B, the authors conclude that “COX Va may interact with Bcl-2 in some way” – this is a very vague speculation. Using only a RT-PCR approach to identify a possible interaction between COX Va and Bcl-2 is just unsatisfying. Most researchers would firstly look via immunoprecipitation for such possible interaction. If the authors will keep their findings regarding Bcl-2 they have to improve their experimental work. Anyway, the authors should know whether they want to focus only on COX Va and its relation to migration, invasion and prediction of distant metastasis or, in addition, to its possible interaction with Bcl-2 which, of course, would strengthen their interesting findings.

→ We thank the reviewer’s suggestions. We agree that immunoprecipitation is mandatory to prove the interaction between COX Va and Bcl-2. However, such work had been accomplished by others previously (reference 34). Therefore, we re-write the last two paragraphs of the discussion section and hope it can facilitate the readers to realize our speculations from our current data.