Author's response to reviews

Title: Mitochondrial Proteomics of Nasopharyngeal Carcinoma Metastasis

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Version: 2 Date: 12 October 2012

Author's response to reviews: see over
October 12, 2012

Dr William CS Cho
Associate Editor
BMC Medical Genomics
BioMed Central
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Dear Dr. Cho,

Many thanks for your editorial decision, the positive comments from the reviewer Dr. Chen, and very helpful comments from the reviewer Dr. Wong. We have taken all comments into account and revised our manuscript. Enclosed are our point-by-point responses to each comment from the reviewer Dr. Wong and editorial requirement. All changes in the text are shown in bold font. I hope that the revised manuscript would be acceptable for publication in the journal *BMC Medical Genomics*.

I look forward to hearing from you.

Thank you.

Sincerely,

Xianquan Zhan

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Response to the comments from the reviewer Dr. Wong and editorial requirement

**Comment 1:** “The authors used the conventional proteomic approach to identify the differential expressed proteins in 2 nasopharyngeal carcinoma cell lines, both of which have the same origin and one has the ability to migrate and metastasis. As the authors intended to use these 2 cell lines as in vitro models to convince that the differential expressed proteins between these 2 cell lines are linked to the cancer cell ability to metastasis, the origin of the cell lines and the methods used to test the metastatic potential should be provided.”

**Response:** The origin of two cell lines has been published in the added
references [1, 2, and 3]. The 5-8F and 6-10B cell lines are two different subcolonies that were originated from the same SUNE-1 cell lines. The tumorigenic ability and metastatic ability of two cell lines were tested by the in vitro colony formation in soft agar and in vivo animal model. The in vitro and in vivo experiments confirmed that 5-8F cells have high tumorigenic ability and 6-10B has tumorigenic ability [2]. In vivo experiment confirmed that 5-8F has high metastatic ability, 6-10B has no metastatic ability [2]. Moreover, the high tumorigenic ability means high cell proliferative ability which is the basis of metastasis (Int J Cancer, 1991, 47: 771; Int J Cancer, 1990, 45: 968). Therefore, 5-8F and 6-10B cells are the excellent in vitro model to discover metastasis-related differentially expressed proteins. The details were added in the lines 88-90 on page 5 of the revised manuscript. Moreover, after the new references 1, 2, 3 were added, all other references were re-numbered and revised in the text.

Comment 2: “The introduction session looks more like a discussion on the selection of experimental models and an explanation on the study design.”
Response: The molecular mechanisms of tumor metastasis remain unclear. A good experimental model and study design are very important to study the molecular event of tumor metastasis. Therefore, we prefer the current style in the section of introduction that fully discusses the selection of experimental model and the study design. Moreover, according to the previously published DEP and DEG data, we modified the corresponding text in the section of introduction.

Comment 3: “The authors mentioned that DEG and DEP has been identified in the 2 cell lines but the functional relationships between the differential gene/gene products are not clarified. In this work, the authors repeated the experiments and produced a large data set with no significant difference with the previous works. If the focus is on the bioinformatics analysis, the authors could gather significantly large amount of data from the public domain. If the DEG data is also available, is there any linkage between the DEG and DEP profile?”
Response: The 78 DEGs [4, 5] and 28 DEPs [6, 7] identified by other research groups were derived from the whole-cell samples. However, those DEGs and DEPs were not fully rationalized in the biological process of NPC metastasis, and there is no any overlap between those 78 DEGs and 28 DEPs. It would result from the following limitation factors: (i) the range of the protein abundance of the whole-cell sample is too wide, not all proteins were extracted; (ii) 2DGE only can array very small partial components of a whole-cell proteome, and (iii) the process of translation of DEG to DEP is regulated by multiple factors, which results in the low consistent rate between the DEG and DEP profiles. The subcellular proteomics such as just focusing on one compartment of a cell would more effectively discover function-related proteins
because the subcellular proteome is much simpler than the whole-cell proteome, it can maximally identify the protein components in a subcellular proteome. Many evidences have demonstrated that mitochondria are the center of oxidative stress, and tumor cell self has anti-oxidant ability for its survival and metastasis. Therefore, a tight relationship exists between mitochondria and tumor metastasis. However, the causes, consequences, and other series of related questions regarding molecular mechanism of mitochondria in tumor metastasis still remain unclear. Thus, we focused on the mitochondrial proteome difference between metastatic 5-8F and 6-10B cells, and provided clues to clarification of the functional role of mitochondria in the process of NPC metastasis. Thus our work was not to repeat the previous other researchers’ work, but to be more in-depth and targeted study. We also did not focus on the bioinformatics analysis of published DEP and DEG data. The corresponding text in the section of introduction was revised.

**Comment 4:** “Attention should be placed on the functional role of the identified DEP in the metastasis of the NPC cells using methods other than just bioinformatics.”

**Response:** Bioinformatics is an effective bridge to link the quantitative data to the biological functions, it provides a clue and direction for further study. We did not just relay on the bioinformatics analysis but after bioinformatics analysis we further used Western blot, siRNA transfection, and Transwell assay to test the functions of those mitochondrial DEPs and their contribution to metastasis.

**Comment 5:** “Further, the linkage between mitochondria and metastasis in NPC is not clarified. The authors promoted to suggest that the mitochondrial gene products are linked to the tumor metastasis. As the 2 cell lines are derived from the same parental origin, the genetic background should be the same theoretically. The authors should explain the difference in their observation supplemented with more laboratory data.”

**Response:** Several important publications confirmed that mitochondrial gene products are linked to the tumor metastasis (see reference 14 and Nature, 2012, doi: 10.1038/nature 11452). Our two cell sublines are the excellent in vitro model to study the metastasis-related molecular event as described in the response to the comment 1. The metastatic ability of two cell sublines was fully confirmed by the in vivo mouse model [see reference 2].

**Comment 6:** “In addition, in order to validate the identified DEP in the clinical settings, the authors should confirm their data in the NPC tissues rather than the cell line alone. The transwell assay could only confirm that NPC cells with altered PRDX3 had different migration rate. The effects on cancer cell metastasis could only be confirmed in animal models.”

**Response:** The comment that the identified DEP data should be confirmed in
the NPC tissues is very good. The present work is only the first step in our long-term research plan. We are collecting the NPC tissues for next step in-depth study. Transwell migration assay is a cell-based migratory behavior assay in vitro. This assay involved a two-compartment system where cells may be induced to migrate from an upper-compartment through a porous membrane into a lower-compartment. After the migrated cells are fixed and stained, the migratory behavior of the cells are then quantified by visual inspection. Cell migration, the movement of cells from one area to another area, is central to achieving function such as the metastasis of tumor. Kleme RL first reported that the high correlation between the in vitro migratory potential of tumor cells and their in vivo invasive properties (J Cell Biol, 1998, 140:961-972), since then this assay has gained widespread acceptance. Therefore, the Traswell-based migration rate is an important index to reflect the metastatic properties. The reviewer comment on the animal model experiment of metastasis is another good point. The animal model experiment has been scheduled in our next step study. The present manuscript is the first step in our long-term research plan, namely just focuses on the in vitro model and experiment, and provides a clue and evidence for our next step in vivo experiments.

**Editorial Requirement:** “- Figure titles: All figures must have a figure title listed after the references in the manuscript file. The figure file should not include the title or number (e.g. Figure 1... etc.). The figures are numbered automatically in the order in which they are uploaded. For more information, see the instructions for authors: http://www.biomedcentral.com/info/ifora/figures.”

**Response:** We followed the figure requirements. The previous Figure 1A and 1B were revised as the current Figure 1. The previous Figure 2A, 2B, 2C, and 2D were revised as the current Figure 2. The previous Figure 7A was revised as the current Figure 7. The previous Figure 7B was revised as the current Figure 8. The previous Figure 8 was renumbered as Figure 9. The previous Figure 9 was renumbered as Figure 10. The numbers of all figures were revised in the text.