Author's response to reviews

Title: "Unique trend" and "contradictory trend" in discrimination of primary synchronous lung cancer and metastatic lung cancer

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Author's response to reviews:

Dear Editor,

Many thanks for your letter (August 29, 2013) about our manuscript " "Unique trend" and "contradictory trend" in discrimination of primary synchronous lung cancer and metastatic lung cancer (BMC Cancer MS: 214162249843037) " and thanks for the referees taking time to deal with the manuscript and giving our suggestions. These suggestions are all valuable and very helpful for improving our paper, as well as the important guiding significance to our researches. According to the referees’ suggestions, we have carefully amended and corrected the related contents in our paper. In addition to the referees’ suggestions, we also have made some small revisions in the manuscript. These revisions will not influence the content and framework of the paper. Revised parts have been highlighted using blue text in the paper. The main corrections in the paper and the responses to the referees’ comments are as following.

Reviewer's report
Title: "Unique trend" and "contradictory trend" in discrimination of primary synchronous lung cancer and metastatic lung cancer
Version: 2 Date: 20 June 2013
Reviewer: William Coleman
Reviewer's report:
This is an interesting manuscript that addresses an important clinical problem in lung cancer diagnosis - distinguishing multiple primary lung cancers from metastatic lung cancer. The authors use an innovative experimental approach and the results are possibly paradigm-shifting.

Comments:
1. The authors utilize three tables to describe the 23 patients included in this study. These tables should be combined into a single table. Each of the submitted tables (Tables 1-3) contain a column for "follow-up" and there are no notations for any patient in any of the tables. Hence, this column should be deleted. Appropriate definitions of the abbreviations used should be included in a footnote (for RML, LLL, LUL, etc).

Thank you for your attention and time to our article and valuable suggestions. Original table 1-3 and 4-7 have been combined in single tables (Table 1 and Table 2). We are sorry for not clear enough to point out the follow-up of all the patients and they were followed up and the status recorded as alive or dead in the Table 1. We are also very sorry for not clear enough to point out what RML, LLL, LUL, etc indicates. So we have explained them in table footnote. We deeply appreciate your carefulness in work. Thank you for your attention and time to our article.

2. Methods - The authors describe the microsatellite markers utilized in the analysis of these cancers, but do not provide a basis for their selection.

Thank you for your attention to our article and valuable questions as well as suggestions.

Mercer et al. conducted a study on lung metastatic carcinoma from head-and-neck squamous cell carcinoma and primary lung carcinoma. They found that analysis on the microsatellite alterations and the deletion sites and manners in genomic DNA of the primary and metastatic cancers was helpful in distinguishing them. So we adopted six microsatellite markers (D2S1363, D6S1056, D7S1824, D10S1239, D15S822, and D22S689) in our study, which are the most representative in the detection of measurable genomic variation. Revised parts have been highlighted using blue text in the paper. We deeply appreciate your carefulness in work.

3. Table 4 shows all the results for 6 polymorphic microsatellite markers for 23 patients and 46 cancers (with patients designated Patient 1 through Patient 23. Tables 5-7 simply reproduce these same results but subdivided by category (like primary and metastatic cancers), with renumbering of patients (so Patient 14 in Table 4 is Patient 1 in table 5). The inclusion of this data in a single table should suffice. Tables 5-7 are directly duplicative and should be deleted.

Thank you for your attention to our article and valuable questions as well as suggestions. Original table 4-7 have been combined in a single table (Table 2).

4. The table of microsatellite data (Table 4) contains plus/minus signs to indicate
detection or absence of alleles. The authors should indicate in the text or table footnote what + versus ++ indicates.

Thank you for your attention to our article and valuable questions as well as suggestions. We are very sorry for not clear enough to point out what + and - indicates. So we have explained them in each table footnote.

5. The text associated with the analysis presented in Table 5 indicates that the tumors in Patient 2 and 7 were labeled T1 and T2 arbitrarily resulting in T2 reflecting the primary and T1 reflecting the metastatic lesion. The methods appear to imply that primary tumors are labeled T1 when known. This needs to be corrected and the tumors relabeled to eliminate confusion in the presentation of the data.

Thank you for your attention to our article and valuable questions as well as suggestions. The multiple neoplasms of Primary and Metastatic Tumors from each patient were designated T1 and T2, the actual temporal order of appearance and lineage relationships between tumors (if any) were not known. Thus, in each patient these neoplasms could represent (i) multiple primary lung cancers, or (ii) derived metastatic lesions.

6. The discussion is excessively long and should be appropriately trimmed for length.

Thank you for your attention and time to our article and valuable suggestions. We have carefully made some revisions in the discussion.

Reviewer's report
Title: "Unique trend" and "contradictory trend" in discrimination of primary synchronous lung cancer and metastatic lung cancer
Version: 2 Date: 8 July 2013
Reviewer: Jin-Yuan T Shih
Reviewer's report:
Major Compulsory Revisions
1. As the authors declared, the results of the manuscript are not conclusive because the number of microsatellite markers is too small.

Thank you for your attention to our article and valuable questions as well as suggestions.

Mercer et al. conducted a study on lung metastatic carcinoma from
head-and-neck squamous cell carcinoma and primary lung carcinoma. They found that analysis on the microsatellite alterations and the deletion sites and manners in genomic DNA of the primary and metastatic cancers was helpful in distinguishing them, suggesting that detections of microsatellite alterations and deletion sites in tumor cell DNA could be used as diagnostic and prognostic markers for multiple cancers. In our study, we wanted to discriminate and analyse the characteristic of multiple synchronous lung cancers and metastatic lung cancers that without relying on collection of normal tissue. So we adopted six microsatellite markers (D2S1363, D6S1056, D7S1824, D10S1239, D15S822, and D22S689) in our study, which are the most representative in the detection of measurable genomic variation. In the next step of the study, we will adopt more microsatellite markers to make the results convictively. We deeply appreciate your carefulness in work. Thank you for your attention and time to our article.

2. The study population was too small to make any persuasive conclusions. Thank you for your attention to our article and valuable questions as well as suggestions.

The incidence of multiple primary lung cancers has been reported to range from 0.7% to 15% of patients with lung cancer. Synchronous lung cancers are rare and rather little is known as for their genetic basis. The incidence of synchronous lung carcinomas is variably reported between 1% and 16%. Among the consecutive patients with primary lung cancer who had undergone a surgical resection between April 2003 and December 2012 at the Department of the Thoracic Surgery at West China Hospital, Sichuan, China, only 13 patients were diagnosed with multiple primary lung cancers. In the next step of the study, we will adopt more patients to make the results convictively. We deeply appreciate your carefulness in work. Thank you for your attention and time to our article.

3. The data presented in the redundant ways. The table 4 had all the data of tables 5, 6 and 7.

Thank you for your attention to our article and valuable questions as well as suggestions. Original table 4-7 have been combined in a single table (Table 2).

4. The terms "unique trend" and "contradictory trend" were not defined in the methods.

Thank you very much for your valuable question. We have defined the terms "unique trend" and "contradictory trend" in the results. The "unique trend" is representative in this study, which means that all alleles corresponding to six microsatellite markers were detected in DNA from primary tumors but were reduced or not observed in DNA from metastatic tumors. In the group of synchronous lung tumor with different histological types, the result showed a "contradictory trend". Some alleles were detected in DNA from primary tumors but were reduced or not observed in DNA from metastatic tumors and other alleles corresponding to six microsatellite markers were detected in DNA from metastatic tumors but were reduced or not observed in DNA from primary tumors.
Reviewer's report
Title: "Unique trend" and "contradictory trend" in discrimination of primary synchronous lung cancer and metastatic lung cancer
Version: 2 Date: 19 July 2013
Reviewer: Mitsuhiro Takenoyama

Reviewer's report:
This manuscript entitled "Unique trend" and "contradictory trend" in discrimination of primary synchronous lung cancer and metastatic lung cancer has reported the usefulness of polymorphic microsatellite markers to distinguish intrapulmonary metastasis from primary lung cancer. This approach and the data are interesting and potentially contain useful information for the readers of BMC cancer but there are several points to be clearly answered before acceptance.

Major remarks:
1. Table 4 should be deleted because of the same content in Table 5-7.
   Thank you for your attention to our article and valuable questions as well as suggestions. Original table 4-7 have been combined in a single table (Table 2).
2. Discussion is too redundant and should be much more concise.
   Thank you for your attention and time to our article and valuable suggestions. We have carefully made some revisions in the discussion.
3. Papers reporting other molecular markers and protein expression in cancer-related genes for the discrimination of primary lung cancer and intrapulmonary metastasis should be added in the discussion section.
   Thank you for your attention and time to our article and valuable suggestions. As the result showed before, Patient 2 provided a clear example of a patient with clonally-related tumors, where the evidence suggest that the T1 metastasized to the T2. In this case, microsatellite markers D2S1363, D6S1056, D15S822 and D22S689 were detected in DNA from T1, but these markers were not found in DNA from T2. The “trend” was similar to the group of primary and metastatic tumors which was considered as intrapulmonary metastasis. The observations of patient 6 and 8 were also representing intrapulmonary metastasis, based upon the “trend”.
4. Tumors of Patient 4 and 5 in Table 7 could be double primary lung cancer from the results of microsatellite pattern. If so, prognosis of these two patients are expected to be better than the other patients. The prognosis of 8 patients would be added in Table 7.
   Thank you for your attention and time to our article and valuable suggestions. We are very sorry for not clear enough to point out the follow-up of all the patients and they were followed up and the status recorded as alive or dead in the Table 1. The patients of case 1,2 and 8 were dead and the others, including patient 4 and 5, were alive.
Minor remarks:
Several mistakes, grammatical errors and spelling miss are found as follows:
On page 1: conquence # consequence
Change made as indicated by the reviewer.
On page 2 in line 26: grammatical error
Change made as indicated by the reviewer.
On page 2: similarity # similar
Change made as indicated by the reviewer.
On page 4: stude # study
Change made as indicated by the reviewer.
On page 8: up # upper
Change made as indicated by the reviewer.
On page 8: low # lower
Change made as indicated by the reviewer.
On page 9 in line4: D22S689#D10S1239
Change made as indicated by the reviewer.
On page 9 in line 11: Table 4#Table 7
Change made as indicated by the reviewer.
On page 9 in line 15: Patient 6 and 9 (Table 4) #Patient 3 and 7 (Table 7)
Change made as indicated by the reviewer.
Microsatellite markers D10S1239 of Patient 5 was reduced in Table 5, however in the text, it was described to be not detected on page 6.
Thank you for your attention to our article. The two neoplasms share common allelic patterns for microsatellite marker at D10S1239 in table 2.