Author's response to reviews

Title: Combined immunohistochemistry of beta-catenin, cytokeratin 7, and cytokeratin 20 is useful for differential diagnosis of lung cancer

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Author’s response to reviews: see over
Dear Professor Peter Newmark:

Our manuscript number is MS 2645390346435566

Thank you for your kind comments concerning our manuscript (manuscript number is 2645390346435566) entitled “Combined immunohistochemistry of β-catenin, cytokeratin 7, and cytokeratin 20 is useful for differential diagnosis between metastatic lung cancer from colorectal cancer and primary lung adenocarcinoma tubular type” by Satoshi Ikeda, Masahiko Fujimori, Satoshi Shibata, Masazumi Okajima, Yasuyo Ishizaki, Takeshi Kurihara, Yoshihiro Miyata, Yosuke Shimizu, Noriaki Tokumoto and Toshimasa Asahara (revised title is “Combined immunohistochemistry of β-catenin, cytokeratin 7, and cytokeratin 20 is useful for the differential diagnosis of lung cancer”).

We appreciate your useful and favorable comments as well as those from the reviewers. We have revised the manuscript thoroughly in accordance with the reviewers’ comments. Our responses to the reviewers’ comments and our revisions in the paper are described in the accompanying sheets.

We hope that our revised manuscript is now satisfactory for publication in “BMC Cancer”.

Sincerely yours,

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Responses to the reviewers’ comments:

Title: Combined immunohistochemistry of β-catenin, cytokeratin 7, and cytokeratin 20 is useful for differential diagnosis between metastatic lung cancer from colorectal cancer and primary lung adenocarcinoma tubular type

(Revised title: Combined immunohistochemistry of β-catenin, cytokeratin 7, and cytokeratin 20 is useful for the differential diagnosis of lung cancer)

Authors: Satoshi Ikeda, Masahiko Fujimori, Satoshi Shibata, Masazumi Okajima, Yasuyo Ishizaki, Takeshi Kurihara, Yoshihiro Miyata, Yosuke Shimizu, Noriaki Tokumoto and Toshimasa Asahara

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The following two major changes have been made to the manuscript: (1) the manuscript title has been changed and (2) figure 2 has been deleted.

Reviewer #1

Major Compulsory Revisions

(1) The reviewer said that (a) the number of patients group is small and the authors should make a comment on the operation statistics on the department (b) needle biopsy should not be mentioned as a separate category.

(a) This comment is the same as comment (3) of Reviewer #2. Statistical data on the operations performed in our department, including the number of lung and colon cancer operations, have been described in the Methods section (page 7, line 3 through 7).

(b) This comment is similar to the Major Compulsory Revisions (6) of Reviewer #1. Figure 2, which displays the immunohistochemical results of a needle biopsy sample from a lung metastasis of colorectal cancer, has been deleted.

(2) The reviewer said that (a) “lung adenocarcinoma of tubular type” has to be introduced by stating the classification system and that the phenotypes of β-catenin and CKs of other subtypes of lung cancer should be described. (b) Furthermore, the reviewer said that it should be discussed that primary lung adenocarcinomas similar in their CK phenotype to colorectal cancer.

(a) The term “lung adenocarcinoma of tubular type” that was used in the manuscript is derived from the old Japanese classification of lung cancer. We have revised “lung adenocarcinoma of tubular type” to “acinar lung adenocarcinoma” based on the classification criteria followed by WHO (1999) (Page 7, line 10). As described by the reviewer, CK phenotypes in lung cancer vary among the pathological subtypes of lung cancer. Johansson reported that the positive rate of CK7 is high in adenocarcinoma and small cell carcinoma, whereas it is low in squamous cell carcinoma (Johansson L: Histopathologic classification of lung cancer: Relevance of cytokeratin and TTF-1 immunophenotyping. Ann Diagn Pathol 2004, 8:259-267). We have added this point to the Discussion section (Page 12, line 10 from the bottom through line 8).

(b) According to the reviewer’s comment, the CK phenotype of primary lung adenocarcinoma is similar to that of colorectal cancer. However, several papers that we have referred to in our manuscript as well as other papers have described that the majority of primary lung adenocarcinoma and colorectal cancers differed in their CK pattern, particularly with regard to in CK7 and CK20. Hence, we have not discussed this further in our manuscript.
(3) The reviewer pointed out that positive rate of β-catenin immunohistochemistry in our study is different from that of other studies. This point has to be discussed in the Discussion section.

We carefully checked the papers of the other groups mentioned by the reviewer in his comments (Hugh TJ, et al: beta-catenin expression in primary and metastatic colorectal carcinoma. Int J Cancer 1999, 82(4):504-511 and Resnick MB, et al: Epidermal growth factor receptor, c-MET, beta-catenin, and p53 expression as prognostic indicators in stage II colon cancer: a tissue microarray study. Clin Cancer Res 2004, 10(9):3069-3075). In these papers, the positive rate of β-catenin immunohistochemistry in the nucleus and/or cytoplasm was 83% and 92%, respectively, in the samples (our results showed a β-catenin positive rate of 100% in 10 samples). The difference in the β-catenin positive rate may be due to the small number of samples in our study; however, we believe that a large difference does not exist between our data and those of others. In accordance with the reviewer’s comment, we have commented on these differences and have included these references in the Discussion section (Page 11, line 7 from the bottom through line 3).

(4) The reviewer said that a better balance is needed in the Discussion between the own finding and the molecular pathology details from references.

In accordance with the reviewer’s comment, we have deleted several details of molecular pathology details from the Discussion section.

(5) The reviewer recommended that description of TTF-1 in the conclusion section should be deleted because the authors did not analyzed TTF-1 immunostaining in the present study.

In accordance with the reviewer’s comment, we have deleted the description of TTF-1 from the Conclusion section.

(6) The reviewer said that Fig 2 is unnecessary. Furthermore, the reviewer pointed out that the reactions to β-catenin have a kind of cytoplasmic background stain in Figs 1A and 1C.

This comment is similar to the Major Compulsory Revisions (1) (b) of Reviewer #1. We have deleted Figure 2. As the reviewer pointed out, several staining intensities of β-catenin were sometimes observed in the cytoplasm of normal cells. We defined positive staining of β-catenin by comparing the staining intensities of tumor cells and the adjacent non-tumor cells as described in the Methods section.

(7) The reviewer said that language editing is necessary because errors are present throughout the text and there are sentences hard to understand. Furthermore, the terminology used in this paper should be changed to the international standards.

We agree with the reviewer’s comments. Abbreviations such as MLC, CRC, and LAT have been spelled out in keeping with the reviewer’s comment. We have carefully corrected spelling and grammatical errors with the help of advice from native speakers.

**Minor Essential Revisions**

(1) The reviewer said that the Title is long and complicated.

In accordance with the reviewer’s comment, we have shortened the title of the manuscript appropriately (page 1, line 1 through 2).
(2) The reviewer said that “Metastatic lung cancer” should be replaced to “Lung metastasis of colorectal cancer”.

We have replaced “metastatic lung cancer” with “lung metastasis of colorectal cancer” throughout the manuscript.

(3) “MLC”, “CRC” and “LAT” are not well-established abbreviations.

We have spelled out these abbreviations. “LAT (lung adenocarcinoma of tubular type)” was replaced with “acinar lung adenocarcinoma” as described above.

(4) The reviewer said that it is difficult to understand the meaning of the first sentence of Background.

We have changed the expression of lung metastasis in accordance with the reviewer’s comment (page 5, line 2 through 3).

(5) The reviewer said that “cell proliferation, morphology and fate” is unusual combination of terms.

In accordance with the reviewer’s comment, we have revised the description of the Wnt signaling pathway (page 5, line 11 through 12).

(6) The reviewer said that it is difficult to understand several explanations in Result section.

In accordance with the reviewer’s comment, we have revised these explanations (page 9, line 4 through 5 and Page 9, line 9 from the bottom).

(7) (8) The reviewer pointed out mistakes.

In accordance with the reviewer’s comments, we have corrected the errors.

Reviewer #2

(1) The reviewer said that there is a mistake in Table 2.

We have corrected the mistake in Table 2.

(2) The reviewer said that how about the intensity and distribution of β-catenin immunoreactivity.

As pointed out by the reviewer, heterogeneity in immunostaining intensity and distribution was observed in the mass. As described in the Methods section, immunostaining results were evaluated by comparing staining intensities of tumor cells and adjacent non-tumor cells, and a positive result was defined as one in which more than 10% of tumor cells were strongly stained by each antibody when compared with adjacent non-tumor cells. However, in fact, more than 50% of cancer cells showed positive staining of β-catenin, CK7, and CK20 in the samples used in the present study.

(3) The reviewer said that the number of the specimen might not be sufficient.

This comment is as the same as the Major Compulsory Revisions (3) of Reviewer #1.