Author’s response to reviews

Title: EphA4 is a prognostic factor in gastric cancer

Authors:

Kohji Miyazaki (k-miyazaki.srg2@tmd.ac.jp)
Mikito Inokuchi (m-inokuchi.srg2@tmd.ac.jp)
Yoko Takagi (takagi.srg2@tmd.ac.jp)
Keiji Kato (keiji-kato.srg2@tmd.ac.jp)
Kazuyuki Kojima (k-kojima.srg2@tmd.ac.jp)
Kenichi Sugihara (k-sugi.srg2@tmd.ac.jp)

Version: 3 Date: 25 March 2013

Author’s response to reviews:

Dear Editor-in-Chief,

BMC Clinical Pathology

We have revised our manuscript (No. 1116638551867708) entitled “EphA4 is a prognostic factor in gastric cancer” on the basis of the reviewer’s comments. We have underlined the revised parts of the manuscript.

To referee 1:

Major revisions

1. All antibodies were used in other studies (Ref #9, 12-14) and the specificities of EphA2 and ephrinA1 antibodies have been demonstrated by immunoadsorption tests or Western blotting (Ref #13, 14). In Ref #9 and #12 studies, the catalogue number of EphA4 antibody available from Santa Cruz Biotech was not specified, but it is only one used for paraffin-embedded tissue. We should have performed the recommended tests in this study, although we cannot afford to do them given the limited time for resubmission. We only treated negative controls using normal rabbit IgG and positive controls in breast cancer tissue in several biological settings. We have described the catalogue number of each antibody and mentioned the specificities as demonstrated in other studies in the Methods section (page 7, lines 10-15 and page 8, line 16).

Positive control of ephrinA1 in breast cancer is shown in Figure 1.

2. We have provided the details of immunohistological scores (intensity + extenty score) for high expression of EphA2, EphA4, and ephrinA1 (Results section, page 10, lines 12-15).

Minor revisions

1. We have shown various expressions of immunostaining in normal and cancerous tissue in Figure 1. In addition, ephrinA in breast cancer is shown as a positive control in Figure 1. The legend of Figure 1 was revised (page 25, last
2. In other studies (Ref #14, 32), ephrinA1 stimulation promoted degradation of EphA2, but increased phosphorylated EphA2 in tumor cell lines. We cannot explain the reason for the discrepancy with the results of our study. However, Ogawa showed positive expression of EphA2 and ephrinA1 in various human tumors (Ref #33), and EphA2 may be controlled by various pathways. We have added Ref #32 to the Discussion section (page 15, lines 15-16). As we have stated, some other studies have shown positive correlations between ephrinA1 and EphA2 (Discussion section, page 15, lines 10-12).

3. In stage II and III disease, there are few T1 and T2 cancers. The difference in DSS between T4 and T1-3 was more statistically significant than that between T1-2 and T3-4 in this population. Therefore, pathological T was classified into T1-3 and T4 only for the analysis of these stage cancers (Results section, page 13, lines 3-6).

4. The discussion on the mechanistic story was shortened as you recommended, and some references were excluded and rearranged.

To referee 2

1. We corrected the numerals in the Abstract section as you pointed out (page 2, line 15, and page 3, lines 2-3).

2. The numerals in Table 4 mean 5-year DSS, not number of patients.

3. As you mentioned, capital letters such as ‘A’ were described for each picture.

4. All antibodies were used in other studies (Ref #9, 12-14), and the specificity was demonstrated (Methods section, page 7, lines 12-14), although we should have confirmed the specificity of each antibody in this study. The discrepancy between primary tumors and metastatic lymph nodes may have been caused by methodological issues. However, gene analysis by extraction from metastatic lymph nodes may be difficult owing to the presence of scattered tumor cells in innumerable lymphocytes (Discussion section, page 17, lines 1-4).

5. As you mentioned, gene expression or quantitative assessment is very important, although mRNA expression levels of EphA2, EphA4, and ephrinA1 were shown to be higher in gastric cancer tissue than in non-cancerous gastric tissue by other investigators (Discussion section, page 16, lines 5-8). The heterogeneity of gastric cancer tissue, including the presence of many stromal cells, may lead to wrong conclusions on analysis of gene expression, because Eph/ephrin was detected in stromal cells. We would like to perform gene analysis using a tumor-dissection system, if such a system is available.

Sincerely yours,

Mikito Inokuchi
Department of Surgery, Tokyo Medical and Dental University
1-5-45, Yushima, Bunkyo-ku, Tokyo, 113-8519, Japan
Phone: +81-3-6831-7009