Author's response to reviews

Title: Clinicopathological significance of platelet-derived growth factor (PDGF) and vascular endothelial growth factor expression, PDGF receptor-beta phosphorylation, and microvessel density in gastric cancer

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Author's response to reviews:

Professor Melissa Norton
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BMC Cancer

Re: Revised manuscript (Manuscript ID 577510343520789)

Dear Professor Melissa Norton;

We are sending herewith the revised manuscript entitled "Clinicopathological significance of platelet-derived growth factor (PDGF)-B and vascular endothelial growth factor-A expression, PDGF receptor-# phosphorylation, and microvessel density in gastric cancer" (Manuscript ID 5775103433520789) by S. Suzuki, et al. Lists of corrections describing all the changes made according to the suggestions by reviewers were indicated point-by-point and were sent to the column of “Covering letter describing revisions in this version of the manuscript”. Changed or added parts were underlined.

Although the volume of the manuscript was slightly increased, this was due to the additional request of descriptions by the reviewer.

We would be grateful if the manuscript could be accepted in BMC Cancer.

Sincerely yours, Shioto Suzuki#M.D.
Department of Molecular and Cellular Pathology,
Reviewer 1: Dr. Yihai Cao
We have responded to the critique from Reviewer 1 as follows:

1. The Reviewer requested to specify the PDGF and VEGF subtype throughout the manuscript including the title.
   -- According to the Reviewer’s suggestion, we have specified the PDGF and VEGF subtypes throughout our manuscript.

2. The Reviewer requested to correlate the hypoxia probe staining with the VEGF-A and PDGF-B expression.
   -- According to the Reviewer’s suggestion, we immunohistochemically stained specimens using HIF-1# antibodies (H1#67, Novus Biologials), which were used in previous reports (ref 22 and 24: BMC Cancer 2008, 8:123.; Oncology, 2007, 72:111-117.). We could not obtain positive correlation between staining results of HIF-1# and VEGF/PDGF. These findings have been added in Results (page 10, line 3-6). The relation between hypoxia and VEGF-A expression is well recognized; the reason why we did not observe simultaneous staining of these proteins in histological sections, may be due to the transient activation by HIF-1# or to multiple inputs and complex cross-talk between molecules and signaling pathways.

3. The Reviewer requested to stain pericytes and correlate the pericyte staining with the malignant disease.
   -- We agree with this comment. As the Reviewer requested, we immunohistochemically stained pericytes using antibodies for #SMA, PDGFR-# and NG2 and have added the findings in Results (page 9, line 24, continuing on page 10, line 1) and representative cases have been shown in Figure 2.

4. The Reviewer requested to make the data much better presented using Tables and graphs in a quantitative manner.
   -- According to this Reviewer’s suggestion, we have added a new table as Table 1 in revised manuscript and have modified Tables 2 and 3 in previous manuscript as Tables 3 and 4 in revised manuscript.

5. The Reviewer requested to add some important publications in this field.
   -- According to the Reviewer’s suggestion, five additional relevant references have been cited (#9-13). Accordingly, the rest of the Reference Numbers have automatically been changed.
6. The Reviewer requested to add some arrows to indicate the important information in histograms.

-- According to the Reviewer’s suggestion, arrows have been added to emphasize findings in Figures 1 and 2.

Reviewer 2: Dr. Katharina Staufer

1. The Reviewer requested to provide new insight into the role of PDGFR/PDGF in gastric cancer, as correlation with survival and other important parameters, and present a representative analysis of at least 10 patients in Western blot methods.

-- We agree with this comment. We checked the clinical data about prognosis, to clarify the association between disease progression and VEGF and PDGF overexpression and other factors. Although a correlation between lymph node metastases and survival was observed, no significant association was found between survival and VEGF or PDGF overexpression, or MVD (Results section, page 10, line 8-10). Moreover, we tried to get representative results of blots in one gel for presentation. However, the fresh tissue from several cases of this study was completely used up, so, unfortunately, we are unable to perform the requested immunoblot experiment. If this referee still regards this data as unacceptable, we will withdraw this part. We will leave the final decision to this referee and Editor.

2. The Reviewer requested to stain for pericyte markers.

-- We agree with this comment. As the Reviewer suggested, we have immunohistochemically stained pericytes using antibodies for #SMA, PDGFR-# and NG2. Positive stainings for PDGFR-# and #SMA were seen predominantly in the membrane and cytoplasm of the stromal cells, including pericytes, although positive staining for NG2 was observed in only a few stromal cells. In detail, PDGFR-# staining was seen more selectively in cells around vessels, whereas #SMA staining was observed also in many other stromal cells. These findings have been added in Results section (page 9, line 24, continuing on page 10, line 1). Representative figures are shown in Figure 2.

3. The Reviewer requested to present the tissue sample as a panel with 5-6 representative patients.

-- According to the Reviewer’s suggestion, the overall results on tissue samples were categorized into 4 types, and representative figures of IHC were shown in Figure 2.

4. The Reviewer requested to move the clinico-pathological factors section in Results to Materials and Methods section.

-- According to this Reviewer’s request, the section about clinico-pathological factors section has been moved to Materials and Methods section (page 4, line 26-35) and a new table has been added as Table 1 in the revised manuscript.

5. The Reviewer requested to present excel files in an adequate fashion to allow
readability.

-- According to this Reviewer's suggestion, excel files have been modified (Tables 1-4).

Reviewer 3: Dr. Nancy Lewis

1. The Reviewer requested to make it clear whether all of the determinations were done by “two independent pathologists” or “one observer”.

   -- As the Reviewer pointed out, “one observer” has been corrected to “two observers” in Materials and Methods (page 6, line 7-8).

2. The Reviewer requested to clarify the term "overexpression", since it was used for PDGF, VEGF and PDGFR but determined by different laboratory techniques.

   -- According to this Reviewer’s suggestion, we have added phrases “overexpression, as measured by a summation score of staining intensity and proportion of positive staining cells” to clarify the term "overexpression" in both Results (page 8, 21-23) and Discussion (page 11, 28-30) sections.

3. The Reviewer requested to make it clear that only two representative samples were shown in Figure 3.

   -- According to this Reviewer’s suggestion, the description of Figure 3 has been corrected to “two representative tissue specimens are shown in Figure 3” in Results (page 10, line 17-18).

4. The Reviewer requested to clarify what the p values actually represent in Table 1.

   -- According to the Reviewer’s suggestion, the description about the relationships between PDGF overexpression and that of VEGF was corrected to “Overexpression of PDGF-B was more common in cases with overexpression of VEGF-A than in those without VEGF-A overexpression” in Results (page 8, line 33-35).

5. The Reviewer requested to make Figure 1. Legend titled as “Microvessel density staining for CD34 in intestinal type gastric cancer”.

   -- According to this Reviewer's suggestion, the legend for Figure 1 has been corrected (Figure 1).

6. The Reviewer requested to correct the label "T" as "C" in Figure 3 legend.

   -- As the Reviewer pointed out, the label “T” has been corrected to “C” in Figure 3 legend (Figure 3).

7. The Reviewer requested to simplify Table 2 and Table 3.

   -- According to this Reviewer’s suggestion, Tables 2 and 3 (in previous manuscript) have been simplified as Table 3 and 4 in the revised manuscript.

Reviewer 4: Dr. Stefan Paul Mönig

1. The Reviewer requested to make it clear why only 109 cases are listed in 7
years (15 per year) (in Patients and Methods section).

-- As the Reviewer pointed out, 109 cases in 7 years is a small number for our hospital. The reason is that we excluded from our study gastric patients who had undergone 1) endoscopic resection, including endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD) because of their limitation of specimens, or 2) any preoperative treatments, including neoadjuvant therapy, because of their possible influence on expression of PDGF, VEGF or PDGFR in specimens. This has been explained on page 4, line 30-35.

2. The Reviewer requested to mention about extent of surgical resection (gastrectomy vs. partial resection), extent of lymphadenectomy (D1 or D2 lymphadenectomy) and residual tumour status (R-category), neoadjuvant or adjuvant therapy.

-- According to the Reviewer's suggestion, this description has been added on page 4, line 30-35.

3. The Reviewer requested to classify the specimens according to the UICC-TNM classification system (T1-4; N0-3).

-- According to the Reviewer's suggestion, description about classification of the specimens has been added in Materials and Methods section (page 4, line 26-28) and new table has been added as Table 1 (in revised manuscript) for the data.

4. The Reviewer requested to mention about postoperative morbidity.

-- According to the Reviewer's suggestion, description about prognosis has been added on page 10, line 8-10.