Author's response to reviews

Title: Role of emmprin in endometrial cancer

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Author's response to reviews: see over
Dear BMC Cancer Editor

Thank you for your letter regarding our paper entitled “Role of emmprin in endometrial cancer” (BMC Cancer).

We have revised our manuscript according to the reviewer’s comments.

**Reviewer’s comments #1:**

**Reviewer:** Katsutoshi Oda

Q1. It is still uncertain whether high emmprin expression might be clinically useful to predict poor prognostic patients with endometrial cancer. First, they did not perform multivariate analysis. Second, they showed that high emmprin expression is associated with almost all the aggressive clinicopathologic factors, including advanced stage, high grade, lymph node metastasis and deep myometrial invasion. According to these findings, high emmprin expression might not be an independent poor prognostic factor, but might rather mirror the aggressiveness which can be easily diagnosed by pathologic findings. Third, the prognosis of high emmprin expression is still favorable. Although it correlates with various types of aggressive morphological findings, the 5 yr-overall survival rate is over 70%. It might be more precise to describe that “LOW emmprin expression might be a predictor of FAVORABLE prognosis”.

A1. We examined independent prognostic factor for disease free survival (DFS) and overall survival (OS) of the clinicopathologic factors including stage, histology, lymph node metastasis, deep myometrial invasion, ovarian metastasis and emmprin by multiple analysis. The ovarian metastasis was strongest independent prognostic factor for DFS and OS by multiple analysis (P=0.0245 and P=0.0222). However, emmprin expression was not an independent prognostic factor for DFS and OS. We have revised our manuscript according to the reviewer’s comments. We changed the conclusions that low emmprin expression might be a predictor of favorable prognosis in endometrial cancer patients, and that emmprin may represent a potential therapeutic target for endometrial cancer.

Q2. Although the authors described that emmprin expression is associated with EMT, they did not include any carcinosarcoma cases in this study. It should be
important to see whether emmprin expression is increased in carcinosarcoma cases (and possibly non-endometrioid adenocarcinomas as type II).

A2. Emmprin expression was examined the patient of carcinosarcoma (n=6) specimens by immunohistochemistry. Moderate epithelial staining was observed in 2 cases (33.3%) and strong staining in 4 cases (66.7%). We add 6 samples of carcinoma, and total cancer numbers are 134 patients. As expected, the expression of emmprin had significant associations with clinicopathological parameters such as FIGO stage ($p=0.009$), histology ($p=0.017$), depth of myometrial invasion ($p=0.001$), cervical involvement ($p=0.001$), lymph node metastasis ($p<0.001$), lymph vascular space (LVS) involvement ($p<0.001$) and peritoneal cytology ($p=0.031$), whereas age and ovarian metastasis did not have significant associations ($p<0.05$, Mann–Whitney U-test). The DFS and OS rates of patients with high emmprin expression (score 2) were significantly higher than those of patients with low emmprin expression (scores 0–1) (DFS: $p<0.001$; OS: $p<0.001$).

Q3. All their data are based on experiments with siRNA for emmprin. It would be more confirmative if exogenous emmprin expression was shown to induce phenotypes involved in migration, invasion and EMT. At least, they should refer other papers and discuss the phenotypes by the introduction of emmprin in cancers.

A3. We have revised our manuscript according to the reviewer’s comments. We add sentences. Emmprin in tumor cells triggers the production or release of matrix metalloproteinases in the surrounding mesenchymal cells and tumor cells, thereby contributing to tumor invasion. Furthermore, the association of emmprin with integrins might be important in signaling through emmprin. Emmprin regulates cell adhesion, invasion, and cytoskeleton reorganization in prostate cancer cells. Bladder, prostate and gastric cancer, after transfection with emmprin siRNA, showed a significant inhibitory effect of cell proliferation and invasion. Emmprin is an upstream inducer of several MMPs and is suggested to be the master regulator of MMP production in disease states such as cancer metastasis. The inhibition by emmprin siRNA mediated migration and proliferation, which led to apoptosis by VEGF receptor-2/VEGF system and the matrix degrading protease to block tumor cell growth and invasion in malignant melanoma.

Q4. Although the expression of emmprin might be low in normal tissues, the low
levels of expression might be still important in various organs. It should be addressed whether knock-out mouse of emmprin has been already analyzed.

A4. We have revised our manuscript according to the reviewer’s comments. We add sentences. Emmprin plays a role in a variety of physiological processes as is evident by the diverse deficiencies detectable in emmprin knockout mice. Knockout mice deficient in the emmprin gene are sterile and show various neurological abnormalities. Emmprin-deficient embryos are also difficult to implant. Emmprin has been found to participate in the cell-surface orientation of monocarboxylic acid transporters (MCTs) to the plasma membrane. Dysfunction of the retina in emmprin-deficient mice is ascribed to the failure of plasma membrane integration of MCTs in the tissue.

Q5. They did not describe treatment protocols of the clinical samples. It should be also important whether the emmprin expression might be associated with chemosensitivity (or radiosensitivity if provided).

A5. Immunohistochemistry of endometrial cancer specimens from chemosensitivity patients revealed increased emmprin expression with clinicopathological parameters such as FIGO stage, histology, depth of myometrial invasion, cervical involvement, lymph node metastasis, LVS involvement and peritoneal cytology. Moreover, high levels of emmprin expression were significantly associated with the DFS and OS rates in endometrial cancer. Interestingly, these findings indicate that low emmprin expression might be a predictor of favorable prognosis in endometrial cancer patients.

Q6. They analyzed emmprin expression in five endometrial cell lines. However, the criteria of high emmprin expression in cell lines are not clear. How can they say that emmprin expression levels are high in HEC-50B and KLE cells without controls? It would be good to include appropriate controls for comparison, if possible.

A6. We agree your comment. However, we can not find emmprin expression of positive control, and we could not perform this experiment yet. And emmprin expression of mRNA on endomeiral cancer cell lines is same results by real time PCR.

Q7. This manuscript contains mainly in vitro studies, NOT in vivo studies. The description of the last paragraph in discussion (page 19, line 4) is not
A7. We have revised our manuscript according to the reviewer’s comments. In summary, this study has revealed a critical role for emmprin in endometrial cancer. The present findings suggest that low emmprin expression might be a predictor of favorable prognosis in patients with endometrial cancer and that emmprin may represent a potential therapeutic target for endometrial cancer.

**Reviewer’s comments #2:**
**Reviewer:** Nao Suzuki

**Q1. More detailed description should be added to Table 1 in the manuscript.**

A1. We have revised our manuscript according to the reviewer’s comments.

According to the reviewer’s comment, we have rearranged the manuscript to follow the suggested order.

We hope that the revised manuscript will now be acceptable for publication in the BMC Cancer.

Sincerely yours,

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