Author's response to reviews

Title: Up-regulated expression of type II very low density lipoprotein receptor correlates with cancer metastasis and has a potential link to beta-catenin in different cancers

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Author's response to reviews: see over
Dear Miss Gorton and Dr Daigo,

Thank you very much for your comments and suggestions. We have revised the manuscript, and responded point by point to the comments as listed below according to the comments and suggestions of reviewers and editors.

I would like to re-submit this revised manuscript to BMC Cancer, and hope it is acceptable for publication on the journal.

Looking forward to hearing from you.

With best wishes

Yours Sincerely

Shen Qu

September 27, 2010

Replies to Editor and Reviewers

First of all, we thank both editors and reviewers for their positive and constructive comments and suggestions. Our responses to the editor’s and reviewer’s concerns are listed below.

Replies to editors:

In this study, all patients who had undergone surgical resection gave informed consent to use excess pathological specimens for research purposes. Research was also carried out in compliance with Helsinki Declaration with the approval of the Ethics Committee of Tongji Medical College (Email: tongjilunli@163.com). According to your advices, the statement to this effect have been added into the part of methods of the revised manuscript (Page 6, line 21), which have been underlined in red color.
Replies to reviewer#1 Hideto Yamada

Thanks for your valuable comments and suggestions!

Major Comments

(1) Is there any relationship between VLDLR II expression and other clinical features including body weight and plasma lipid profiles (such as triglycerides and so on).

Answer: We really neglected the relationship between VLDLR II expression and some clinical features including body weight and plasma lipid profiles. We understand Dr. Yamada’ point, and accept the suggestion. Thus, we consulted the medical records of cancer patients again, and analysed the relationship between VLDLR II expression and additional clinical features (such as body weight, triglycerides, and total cholesterol). Please refer to Table 2, Table 3 and Table 5 in the revised manuscript. The results showed that VLDLR II expression in cancer tissues was not significantly associated with body weight, total cholesterol and triglycerides. Maybe the lipid content are impacted by comprehensive factor.

Generally in our hospitals, the plasma lipid routine examinations do not include LDL-cholesterol and HDL-cholesterol detection. Furthermore, in the process of this study, we did not make the patients do special examinations including LDL-cholesterol and HDL-cholesterol, so we are terribly sorry that we could not provide the data concerning relationship between VLDLR II expression and plasma lipid profiles such as LDL-cholesterol and HDL-cholesterol.

(2) Dose VEGF expression in cancer tissues correlate with VLDLR II expression and distant metastasis.
These representative immunohistochemical photomicrographs of VEGF staining. Comparison of expression of VEGF in normal, undistant metastasis and distant metastasis cancer samples (gastric, breast and lung cancer); original magnification $\times 200$

We accepted the reviewer ’s valuable suggestion and conducted a preliminary experiment on the expression level of VEGF via IHC in gastric, breast and lung cancer samples. As shown in Figure above, VEGF staining is more abundant in distant metastasis cancer tissues. Then we statistical analysed the correlation between VLDLR II and VEGF expression in distant metastasis cancer samples, the results were summarized in Table below, which indicated that the presence of a positive correlation between VLDLR II and VEGF was obtained in 9 breast cancer tissues
with distant metastasis.

It is generally considered that increased VEGF expression enhances vascular permeability and angiogenesis, and is the cause of tissue edema as well as tumor and metastasis formation [1, 2]. We believe that the relationship between VLDLR II and VEGF expression, angiogenesis in cancer is a very important topic, which could further intensify the knowledge of the association with VLDLR II and cancer progression. Thus, we would like to carry out a separate but more extensive experiment on this topic. Thanks for your excellent advice again. Accordingly, we have added the sentence to address the reviewer’s point in the part of discussion of the revised manuscript (page 17, 18; underlined in red color).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Gastric cancer (7)</th>
<th>Breast cancer (9)</th>
<th>Lung cancer (11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VEGF</td>
<td>VEGF</td>
<td>VEGF</td>
</tr>
<tr>
<td>VLDLR II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>0.476</td>
<td>0.675</td>
<td>0.399</td>
</tr>
<tr>
<td>P</td>
<td>0.280</td>
<td>0.046*</td>
<td>0.224</td>
</tr>
</tbody>
</table>

VEGF indicates Vascular endothelial growth factor; R, correlation coefficient; *P< 0.05 was considered statistically significant.

**Minor comments**

(3) The explanation for TNM stage and Tumor size in Table 2-4

**Answer:** We are sorry that we did not explain clearly about T1-T4 in Table2, 3 and 4 in original manuscript so that reviewers had been confused by the classification regarding TNM stage and Tumor size in the tables. By our original intention, T1-T4 indicated TNM stage I -IV in original version of manuscript. We had revised T1-T4
to TNM stage I - IV in Table 2, 3 and 5 in the revised version of the manuscript.

The Pathologic tumor, lymph node, metastasis (TNM) status were assessed in all patients according to the TNM classification system of the UICC/AJCC. In addition, we divided patients into subgroup with Tumor size referring to TNM classification system, and combining with the average value of all patients’ tumor sizes. For example, according to breast cancer TNM classification, tumor size 2.0 cm is the critical value, and in this study the average value of tumor size in all breast cancer tissues is 2.23 cm, so we divided the patients into Tumor size subgroup according to tumor size 2.0 cm in Table 3.

Replies to reviewer#2 Takumi Yamabuki

Thanks for your constructive advices!

Major Compulsory Revisions

(1) The author should show the pathological diagnosis of lung cancer in the appropriate part of manuscript.

Answer: We agree with Dr. Yamabuki that show the pathological diagnosis of lung cancer in the appropriate part of manuscript, and further analyse the differential expression of VLDLR II in tumor and adjacent non-affected tissue in different histological types of lung cancer. There are 4 histological types of lung cancer which related to this study, including squamous cell carcinoma, adenocarcinoma, small cell carcinoma and adeno-squamous carcinoma. The expression of VLDLR II was significantly elevated in tumor tissues in comparison to the corresponding adjacent
non-affected tissues in adenocarcinoma, whereas in other histological types, it was not.

Please refer to Table 4 and Table 5.

(2) beta-catenin expression didn’t correlate with VLDLR expression in lung cancer, how about a subgroup analysis by pathological diagnosis grouping?

**Answer:** According to your advices, we have assessed the correlation between VLDLR expression and beta-catenin expression detected by Western blot in 4 histological types of lung cancer (18 adenocarcinomas, 24 squamous cell carcinoma, 6 small cell carcinoma and 3 adeno-squamous). The results indicated that none of the subgroups showed any significant correlation between VLDLR and beta-catenin expression (Spearman test: \(r = 0.389, P = 0.111\); \(r = 0.102, P = 0.636\); \(r = 0.686, P = 0.872\); and invalid data; respectively).

(3) The author should check and remake the Table 3.

**Answer:** We are sorry that we did not explain clearly about T1-T4 in Table 2, 3 and 4 in original manuscript so that reviewers had been confused by the classification regarding TNM stage and Tumor size in the tables. By our original intention, T1-T4 indicated TNM stage I - IV in original version of manuscript. We accepted Dr. Yamabuki’s comment, and had revised T1-T4 to TNM stage I - IV in Table 2, 3 and 5 in the revised version of manuscript.

(4) How was the expression of full-length VLDLR in the clinical samples of gastric, breast, and lung cancer? Are there any data that you have examined? Are there any studies about it? The author should discuss this point.

**Answer:** Actually, in this work, the expression of full length VLDLR protein were
also detected by Western blot in gastric, breast and lung cancer samples, as shown in Figure below, the protein were found at a low level in cancer samples. In addition, the results indicated that the expression of full-length VLDLR in cancer tissues and matched adjacent normal tissues were of no significant difference in the above-mentioned three kinds of cancer.

It was generally accepted that full-length VLDLR was the major receptor in binding and internalizing lipoprotein enriched in apolipoprotein E (apoE) [3]. Ensler K et al reported that the expression of full-length VLDLR has a significant increase after adipocytic 3T3-L1 cells were induced to differentiate into adipocyte-like cells, this is beneficial for adipocyte to absorb triglyceride [4]. So the full-length VLDLR played an important role for apoE uptake and metabolism. In fact, in our lab, the main research is to reveal the various roles of VLDLR. In previous study, we had shown that over-expression of the full-length VLDLR could not promote SGC7901 cell migration and proliferation, in contrast, when the VLDLR II were transfected into SGC7901 cell, increased the migration and proliferation [5]. In present study, we obtained similar results in tissue levels, the expression of full-length VLDLR in cancer tissues and matched adjacent normal tissues were of no significant difference in gastric, breast and lung cancer samples. And so, the VLDLR II were paid more attention in this study.

Thank you very much for the suggestion, these comments were added into the part of discussion of the revised manuscript (Page 15, line 2; underlined in red color).
**Figure** Expression of full length VLDLR in different cancer samples by Western blot analysis with special antibodies. A: Western blot analysis demonstrated the protein level of full length VLDLR in gastric, breast and lung cancer tissues (T) and matched adjacent normal tissues (N) from 9 representative patients. β-actin served as protein loading control; B: Student t-test analysis with relative protein level of full length VLDLR in gastric, breast and lung cancer patients (25, 23 and 31, respectively).

**Minor Essential Revisions**

(1) The author should correct spelling mistakes.

**Answer:** According to your suggestion, We have corrected spelling mistakes. Please refer to page 6, line 20 and page 11, line 9, which have been underlined in red color.

**References**


2. Carmeliet P: VEGF as a key mediator of angiogenesis in cancer. *Oncology* 2005, 69 Suppl 3:4-10

3. Takahashi S, Kawarabayasi Y, Nakai T, Sakai J, Yamamoto T: Rabbit very low
