Author's response to reviews

Title: Up-regulation of CLDN1 in gastric cancer is correlated with reduced survival

Authors:

Lars L Eftang (lars.eftang@medisin.uio.no)
Ying Esbensen (yinge@medisin.uio.no)
Tone M Tannæs (t.m.tannas@medisin.uio.no)
Gustav P Blom (gustav.peter.Blom@ahu.no)
Ida R K Bukholm (i.r.k.bukholm@medisin.uio.no)
Geir Bukholm (gebu@ous-hf.no)

Version: 3 Date: 21 September 2013

Author's response to reviews: see over
Dear Ms Patricia Ratin and Prof Daiming Fan

Thank you very much for the thorough review of our paper and valuable feedback. Most of the issues from the reviewers have been resolved, however there are some issues regarding the study population size and the further experiments where we are unfortunately unable to conform to one referee’s concerns. Please read our point-by-point response to the reviewer’s comments:

**Referee 1. Reviewer’s report**

**Title:** Up-regulation of CLDN1 in gastric cancer is correlated with reduced survival

**Version:** 2  **Date:** 3 August 2013

**Reviewer:** Shaolei Lu

**Reviewer’s report:**

This study reported transcription profiles of 20 gastric cancers of various pathological stages, lymph node status and histological types. The finding of claudin 1 within the top differentially upregulated genes in gastric cancer echoes another similar study by Cho et al (Clin Cancer Res; 17(7); 1850–7) where claudin 1 is the top one in the upregulated gene list (accessed through oncomine.org). The study also identified a chemokine, IL-8, expressed strongly in gastric cancer. The survival analysis further emphasized the role of claudin 1 in the disease process. Overall, the study is a sound and coherent work.

No major compulsory revision.

Minor essential revisions:

1. Similar gene expression and immunohistochemical studies have been published. Some of them should be mentioned in the discussion, such as the study above and an IHC study (Hum Pathol. 2005 Aug;36(8):886-92).

   We have read the study by Resnick et al (Hum Pathol. 2005 Aug;36(8):886-92) with great interest, and agree that this work adds valuable information to the discussion, and have updated this chapter of the paper accordingly. The study by Cho et al (Clin Cancer Res; 17(7); 1850–7) identified six genes which predicted gastric cancer relapse. CLDN1 was not among these genes and is not mentioned in the paper, however CTNNB1 (beta-catenin) was the most differentially regulated gene in this study. We have therefore only mentioned this paper briefly in the introduction of our manuscript.

2. Intestinal type and diffuse type gastric cancers apparently take two different pathways of tumorigenesis. It is assumed that this study did not reveal any significant gene expression difference in
different cancer types. It is still worth mentioning in the discussion this negative finding to help future researchers design related new studies.

We have performed further analysis on our dataset of filtered genes. On hierarchical clustering of all the tumor and normal mucosa samples, mixed type, intestinal type and diffuse type of gastric cancer formed separate clusters. We have updated the Results chapter and figure 3 with these results, and we discuss these findings in a separate paragraph in the Discussion.

3. The finding of IL-8 highly expressed in cancer tissue could be due to peritumoral or intratumoral chronic and acute inflammation which should be evaluated or commented. We agree with this comment and have updated the Discussion chapter accordingly.

4. Two cancers are from Asian patients. Are they the same two showing active H. pylori infection? It may be mentioned in the text.

The H. pylori positive patients were both Caucasian. We have updated the Results section to clarify this relationship.

Discretionary Revisions:
Good quality claudin-1 antibody is available. It would be useful to show some representative IHC images of claudin-1 in cancers of this study.

Our purpose was to perform a hypothesis-generating study, examining gene patterns that could be associated with cancer survival. The gene expression pattern consists of several genes that are up-regulated or down-regulated. Claudin-1 is only one of these genes. Our results will of course be followed up in further studies with focus on claudin-1 gene expression and claudin-1 protein. It was not the intention of the present study to go into specific detail concerning one single protein. If the editorial board is of another opinion, we can reconsider, but this will imply a significant extension of the present study and will require considerably more time.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.

Referee 2. Reviewer's report
Title: Up-regulation of CLDN1 in gastric cancer is correlated with reduced survival
Version: 2 Date: 16 August 2013
Reviewer: junyan tao
Reviewer's report:
There are some suggestions about minor revise on the paper detailed as following,
1. Some parts of the abstract are not clear, such as the line 4 of the results was not certain. Meanwhile, the abstract doesn’t describe method clearly, so this part needs to be revised. The abstract has been updated with a Methods paragraph, and we have revised line 4 and clarified other parts of the abstract.

2. Some writing is not so good, for example, the first sentence of background which marked by yellow color, and other placed marked by yellow color also need the author to revise. We have clarified the sentences marked in yellow and revised significant parts of the entire manuscript to make it more concise.
3. In introduction part, the author only talked about 2 types of GC, why not talking about the mixed type, and what is the type that the researcher studied in the paper? We have revised the introduction to mention mixed type GC, and clarified throughout the manuscript which types are being studied.

4. In immunohistochemistry part, what’s the antibody dilution concentration used in the paper? The antibody dilution is 1:200 and the Methods chapter has been updated to include this information.

5. In table 1, is there exist difference between male and female in clinicopathological features We have updated table 1 with more detailed information about the patients included in the study and the sex distribution among them.

6. The labels of Figure1 and figure 2 are not clear; please revise to make it better understood. The figure texts have been expanded to improve the explanation the figures.

7. About the discussion part, I think the paragraph “CLDN2 and CLDN7 were among the most up-regulated genes in the tumor samples, and like...... ” is not necessary for this paper. And some other parts are complex and confused, please revise to make it concise and clear. We agree that these findings are not central to the paper, and have summarized, compressed and clarified this part of the Discussion.

8. All the tables need to be revised according to the standard table format of 3 lines. We have read the “Instructions to authors” on formatting of tables (http://www.biomedcentral.com/bmccancer/authors/instructions/researcharticle#preparing-tables), as well as studied tables in recent publications in BMC Cancer. We have reformatted the tables using the 'Table object' in Microsoft Word 2007, to align the columns of data. Visible, black lines are used as borders of each cell, as requested in the on-line instructions.

9. The paper only detailed the up-regulated and down-regulated genes, to CLDN1 and IL-8, the author should better to verify the changes by other methods, such as real-time quantitative real time PCR, or western blot. Our purpose was to perform a hypothesis generating study, examining gene patterns that could be associated with cancer survival. The gene expression pattern consists of several genes that are overexpressed or suppressed. Claudin-1 and IL-8 are only two of these genes. Our results will of course be followed up in further studies with focus on claudin-1 gene expression and claudin-1 protein. It was not our intension to go into these details concerning specific proteins in the present study. If the editorial board is of another opinion we can reconsider, but this will imply a significant extension of the present study and will require more time.

10. The reference parts need to be changed in according to the journal format. We have read the “Instructions to authors” on formatting of references (http://www.biomedcentral.com/bmccancer/authors/instructions/researcharticle#formatting-references), as well as studied the referencing and bibliography in recent publications in BMC Cancer. We have used the http://www.biomedcentral.com/download/refman/biomedcentral.os style file for Reference Manager 12, and we believe the referencing in the text and the bibliography is now according to the correct journal format.

Meanwhile, there are some major revisions as below,

1. The author need to identify what kind of GC with immunohistochemistry is staining studied in the paper.
The Lauren classification of gastric cancer is based on histopathological findings. The specimens have been studied by two separate senior pathologists to verify diagnosis and classification. We believe further classification of the tumor types with immunohistochemistry is not necessary.

2. Another problem is that the clinic patient numbers are too small, since the research is related to GC prognosis, it better has many more patients numbers to verify the biomarker effectively. We completely agree that the study number is small, and we acknowledge this in the discussion and in the conclusion. A larger, prospective study is certainly necessary to verify the prognostic value of CLDN1. Gastric cancer is a relatively rare disease in Norway with its limited population compared to endemic gastric cancer countries. Large clinical studies comparable to the size of some Asian studies would be impossible to conduct in Norway. There is very little published Scandinavian gastric cancer research, and although the patient number is small, we believe our results are interesting.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I declare that I have no competing interests

---

**Referee 3. Reviewer's report**

**Title:** Up-regulation of CLDN1 in gastric cancer is correlated with reduced survival

**Version:** 2 **Date:** 17 August 2013

**Reviewer:** Jie Liang

**Reviewer's report:**

In this study, Lars L Eftang et al used paired tumor and control mucosa for microarray for identification serials of differential expressed genes. And further proved that CLDN1 was up-regulated in gastric cancer, which was independently associated with poor post-operative prognosis value. IL-8 and CLDN1 may represent central links between the gene responses seen in acute H. pylori infection of gastric epithelial cells, and ultimately gastric cancer.

**Major comments:**

Research on CLCN family and GI cancer has been widely investigated. As the paper cited, previous studies have shown that relationship between CLCN1 and gastric cancer been controversial. Like following:


(2) Claudin-1 has tumor suppressive activity and is a direct target of RUNX3 in gastric epithelial cells. (Gastroenterology. 2010 Jan;138(1):255-65.e1-3.)

(3) The expression of claudin-1 was significantly lower in cases of intestinal type adenocarcinoma based on the Lauren classification. (J Surg Res. 2011 May 15;167(2):e185-91)

This study based their work mainly on the results from microarray. However, what’s the reason between different results on CLCN1 and gastric cancer is still unknown.

I would suggest the authors further investigate the possible reason and link between CLCN1 and gastric cancer with subgroup analysis, like according to Lauren classification, Hp infection, and different stage et al. Then we are looking forward to get a clue why relationship between CLCN1 and gastric cancer is different.

The first two papers (ref 32 and 33 in our originally submitted manuscript) were mentioned in the original discussion, and we have read the third paper by Jung et al (J Surg Res. 2011 May
15;167(2):e185-91) with great interest. We agree that this paper adds valuable discussion points, and have revised major parts of the Discussion accordingly. We identified clustering of the different histological subtypes in the gene expression profiles (figure 3), however with CLDN1 there was no significant difference between histological types and the gene expression. We have emphasized this in the Discussion. We have expanded further on the relationship between CLDN1 and gastric cancer in the Discussion.

Minor comments:
English grammar needs to be polished by a native English speaker. Some portions of the paper are poorly written. The whole manuscript contains many grammatical errors. We have proof-read the entire paper, and the manuscript now has a higher quality English and grammar.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Needs some language corrections before being published
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests:
'I declare that I have no competing interests'

We are looking forward to hearing from you.

Best regards,

Lars Lohne Eftang