Author's response to reviews

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

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

Thank you very much for the review of our resubmission. While reviewer 1 and 2 have no specific concerns, reviewer 3 has justified concerns about our findings with regards to CLDN1 levels and histological subtypes, and refers to Jung et al’s work, published in *J Surg Res* 2011[1].

The aim of the present study was to perform a hypothesis-generating study, examining mRNA gene expression patterns associated with cancer survival. It was not our purpose in the study to distinguish between expression patterns within different histopathological subtypes. If that had been the purpose, the design should have been different. As we have pointed out in the Discussion the material is too small to allow comparisons of expression patterns between the subtypes. It is also difficult to directly compare mRNA expression with protein expression. There are often several regulatory steps from mRNA to protein, and it is difficult to draw conclusions about differences between studies that have used mRNA and proteins as different end-points.

However, we have commented on two studies that have examined the protein expression of claudins. These are the studies of Jung et al[1] and of Resnick et al[2]. Both studies have very different designs from our study in that gastric cancer specimens were examined by immunohistochemistry to detect pre-defined proteins: claudin 1, 2, 3 and 4. The two studies came to different conclusions regarding Claudin 1 expression in the gastric cancer subtypes. Resnick found moderate to strong (+2 to +3) staining of claudin 1 in 74% of the intestinal type and only in 46% of the diffuse type, whereas Jung et al found the highest Claudin 1 expression in the diffuse type. Even these two studies are not comparable with regard to methodology as Jung et al has converted claudin staining to positive (+) or negative (-) for “statistical purposes”, on the basis of which the statistics have been calculated, whereas Resnick has used the graded results and compared the relative increase or decrease in staining to matched normal mucosa.

In our study, we have reported the ratio of the CLDN1 gene expression (mRNA) in the tumor and matched normal tissue, to account for variances in background expression between individuals. We feel this is an accurate representation of the relative increase or decrease in any gene expression which may occur in the tumor. Although a small study population, we have performed statistics on
the CLDN1 ratios and histological subtype on all the samples. We stated in our previous manuscript that “We found no significant difference between CLDN1 expression and histological subtypes”. We would like to clarify this: The average CLDN1 ratio (tumor/normal) was higher in the intestinal cancer group (value 3.0) compared to the diffuse type group (1.82). This could support the findings of Resnick (if there is a correlation between mRNA and protein), but contrasts Jung’s study. Nevertheless, our data was not statistically significant (independent t-test), possibly because of the sample size and the manuscript has been updated to clarify this matter. Furthermore, we have also analyzed whether there was a relationship between the absolute CLDN1 values in the tumors (not the tumor/normal ratio), and histological subtypes. Interestingly, although not statistically significant, this difference was much smaller than the difference between the CLDN1 ratios mentioned above, (CLDN1 values: intestinal 9.8, and diffuse 8.75). We believe that reporting the non-significant results in the manuscript would not be correct.

In conclusion, we believe we have performed a good hypothesis generating study that points to mRNA expression of claudins as interesting in gastric cancer pathogenesis. The relationship between mRNA expression and protein expression and the mRNA expression related to different subtypes has been beyond the scope of our present study and should be subject to new studies.

References


We are looking forward to hearing from you.

Best regards,

Lars Lohne Eftang