Author's response to reviews

Title: A proteomic strategy to identify novel serum biomarkers for liver cirrhosis and hepatocellular cancer in individuals with fatty liver disease.

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Version: 2 Date: 27 May 2009

Author's response to reviews: see over
Dear Editors

Thankyou for reconsidering our original paper,

MS: 1141023832246670
A proteomic strategy to identify novel serum biomarkers for liver cirrhosis and hepatocellular cancer in individuals with fatty liver disease.

for publication in ‘BMC Cancer’. We believe our manuscript has been significantly improved following the peer review process and the additional studies performed in order to address the reviewers concerns. A point by point response to each of the reviewers is included. The reviewers comments are in black and underlined. The responses are in red. We hope you now find our article suitable for publication.

Yours sincerely

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27th May 2009
Point by point response to reviewers comments for:

MS: 1141023832246670
A proteomic strategy to identify novel serum biomarkers for liver cirrhosis and hepatocellular cancer in individuals with fatty liver disease.

Reviewer 1
Version: 1 Date: 3 February 2009
Reviewer: Kaichun Wu
Reviewer's report:
Major points
1. In the result part of spot identification, the authors should show the MALDI-TOF MS spectra, especially that for spot 5. Spectra, signal/noise ratio as well as more information on the peaks should be given to strengthen the results. Additionally, the Mascot scores for the identified proteins to judge the quality of the protein identification procedure should be given.

The results sections have been updated to provide this additional information in the form of Table 2. The MALDI-TOF MS spectra and protein summary report for each spot are provided as supplementary information, as is additional information discriminating spot 3.

2. In Figure 2, data from patient group of pre-cirrhotic NAFLD should be added to make the conclusion more convincing.

This figure has now been modified to include data from patients with pre-cirrhotic NAFLD.

3. In Figure 3, sample number seemed too limited to make the conclusion that CD5L does not increase incrementally with the level or stage of fibrosis. More cases should be included to make the conclusion more solid.

We do not have more cases with biopsy staged NAFLD and serum to analyse. However, we have analysed CD5L mRNA expression in and additional 21 pre-cirrhotic liver NAFLD biopsy samples. There was no significant association with fibrosis stage in these tissues. In addition, we have studied CD5L mRNA expression in ‘non-tumour liver’ from 14 patients undergoing liver resection for benign, primary or secondary malignancies. This data is presented in the manuscript as Figure 4.

Minor point
1. The form of Table1 should be changed according to the standard pattern.

This has been changed
Reviewer 2
Reviewer: Jie Liu
Reviewer’s report:

Major Compulsory Revisions
1. about specificity:
Table 1 showed that AFP level was much higher in the patients with cirrhosis plus HCC than with only cirrhosis, how did this Phenomenon reflect in your 2-D results? CD5L has been reported up-regulated in several diseases such as atopic dermatitis, HCV infected HCC, etc. what is the advantage of CD5L compared with AFP as a serum biomarker?

The serum samples selected for 2-D page were those in which AFP was not elevated as we aimed to identify a marker relevant to patients in whom none was available. AFP was therefore not detected in the serum separated by 2-D PAGE. Subsequent CD5L analysis did include all patients.

An elevated AFP has value as a biomarker of relatively advanced HCC and only in up to 60% of patients. We had hoped that CD5L may be complimentary, as a surveillance marker in AFP –ve individuals, and at an earlier stage. This was not shown to be the case, as it was significantly elevated in both cirrhosis cases and cases with cirrhosis+HCC, and did not satisfactorily distinguish the two. There is no added value in individuals in whom AFP is already elevated. Individuals with a normal AFP and a particularly high CD5L may have an HCC, or may be at a high risk of developing one. This is summarised in the discussion.

- Minor Essential Revisions
1. Can CD5L been detected in normal people? How about its sensitivity as a diagnostic biomarker? The results would be more convincing if there are some tests (at least western blotting or PCR) between normal and abnormal cases.

We do not have serum from a cohort of normal individuals with confirmed normal liver histology on liver biopsy. The individuals with a very low risk of developing liver cancer in this cohort are the individuals with simple steatosis on liver biopsy – they have no inflammation, fibrosis or cancer and have relatively low levels of serum CD5L protein. ELISA is more sensitive than western blotting for serum quantification and has been employed in preference to western blotting.

In those cases studied by ELISA, we do not have tissue for PCR or mRNA studies. However, we have attempted to address the important issue of showing a difference between ‘normal’ and ‘abnormal’ cases by means other than ELISA assay. We have studied the CD5L mRNA expression in liver biopsy tissues from 21 NAFLD patients. Unfortunately, it was not possible to obtain sufficient yield of RNA from any cirrhotic biopsy tissues. Instead, we have included a second set of 14 tissues collected at the time of liver resection. Only one of these cases had an underlying cirrhosis and an HCC – resection is rarely performed for HCC in patients with NAFLD. The remainder were ‘normal’ liver tissues obtained at the
time of resection for benign or metstatic tumours (most often colorectal metsatases). This data is presented as Figure 4. We hope, despite small numbers, that the reviewer is convinced that CD5L is increased in NAFLD tissues versus normal livers. Although we have only one case of cirrhosis and HCC in this tissue set, in combination with the ELISA assays in significantly more numbers of patients, we hope this is sufficient to satisfy reviewer 2.

- Discretionary Revisions
1. The alternative expressions of 5 spots in 2-D gel electrophoresis would be more clear if data of MS analysis been added (figure 1).

This has been provided in response to requests from both reviewers.