Author's response to reviews

Title: Modulations of Cell Cycle Checkpoints in HCV Associated Disease

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Version: 3 Date: 24 April 2009

Author's response to reviews: see over
April 24, 2009

To,
The Editor
BMC Infectious Diseases

Re: Revised submission of MS: 5162708601865476 - Modulations of Cell Cycle Checkpoints in HCV Associated Disease

Dear Editor,

We are thankful to the reviewers for their constructive feedback and where possible, have incorporated changes as per their suggestions. Please find below our response to each of the comments made by the two reviewers. Reviewers’ comments are in italic with answers below.

Revisions:

1. The authors performed a general screening on the cell cycle associated genes and some of the genes were up or down-regulated significantly. As the mRNA expression levels are not always associated with the protein expression levels and the proteins are the most important part in exerting the biological functions, you should confirm the protein expression levels on those genes.

   The evaluation of all differentially expressed genes at protein level was technically not feasible. There was a limited amount of total proteins extracted from freshly frozen liver biopsies as well as the number of sections obtainable from formalin fixed liver biopsy blocks were also limited. Some genes considered to play crucial roles in the cell cycle control were, however, analyzed at protein level. Those data are included.

2. It is not clear that why you perform the IHC only on p15 and p27. mRNA expression levels are higher in GADD45A, RAD1 and HUS1.

   The submitted manuscript describes our study that is an extension of our previous observations on HCV-mediated changes in the cell cycle regulators. Previous studies including our own have shown an association of p21 with HCV associated disease. p27 and p15 belong to same CDK inhibitors family as p21; therefore, we were interested in evaluating any association of these differentially expressed CDK inhibitors with HCV disease (Also, see response to ‘1’).
3. Please put the normal control (Figure 1) in the main document of manuscript, it is hard for readers to follow. And according to the data, the expression levels of p15 and p27 are all up-regulated both in early and advanced HCV infection. Why can't we find significant difference between advanced and normal on p27. Moreover, p15 should express at higher level in advanced than early. I do not see any correlation between the IHC data and the mRNA expression data.

As per reviewer's suggestion normal control is now included in Figure 1. The expression levels of p27 in advanced HCV (median 17.3%, range = 3.0 – 32.0) was also significantly high when compared to uninfected normal liver (median 4%, range = 1.3 - 6.3) and this has been included in result section (Page 11) as well. In case of p15, the possible explanation for the difference in IHC and mRNA expression data has been included in Discussion section (Page 14).

4. In figure 3, the protein concentrations are not the same. You should perform it again.

As we were dealing with liver biopsies and only a fraction was available to us for protein estimation. Therefore, we had very limited amount of protein available from liver biopsy sections. This limitation made it impossible to quantitate protein expression and load equal amounts of proteins in each well. However, densitometry analysis was done to measure band intensities and expression of all proteins was normalized to their respective β-actin control, to correct for loading errors. This is stated in the figure legend.

5. The introduction and discussion sections are too long and contains too much information. It is very hard to follow.

These sections have been rechecked and excessive information has been removed.

6. There many grammer errors and typos throughout the manuscript. You should find a native English speaker to edit your manuscript.

The manuscript has been reevaluated to correct grammar and syntax errors.

On behalf of all authors I would express my sincere thanks to you for considering this manuscript.

Yours sincerely,

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