Author's response to reviews

Title: Autophagy and apoptosis-related genes in chronic liver disease and hepatocellular carcinoma

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Author's response to reviews: see over
Dear Editor

enclosed please find the revised version of our manuscript, entitled “Autopgagy and apoptosis-related genes in chronic liver diseases and hepatocellular carcinoma”. The paper has been modified, the number of patients studied increased and we tried to answer the referee’s criticisms, adding additional figures, when required.

We hope that in this revised version the paper is now eligible for publication in the BMC Gastroenterology.

Looking forward to hearing from you.

Yours sincerely,

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Point to point answer to Referee n 1

Major Compulsory Revisions

n. 1 As requested we increased the number of patients including other additional 8 patients with HCC to reach a total number of 93 patients investigated. I agree on the fact that an independent cohort could validate the results obtained but the only group with relative small number of patients is the one of cirrhotic not harboring HCC and it is relatively rare to have biopsies performed in this type of patients. On the other hand 49 patients with CH are included and a total of 42 samples from patients with HCC are examined.

n.2 I agree with the Reviewer that what has been observed in humans must be verified in cell lines. Same experiments have been conducted in HepG2 and HepB cell lines stimulated for different time and with different doses of PIK inhibitors while other experiments are in progress in order to verify what found in human liver biopsies. Preliminary experiments showed the same results found by Takahara T et al. about the Bcl-2 expression in HepG2 and Hep3B cell lines. This however in our mind is a totally different approach, our report being essentially a human study that describes expression of autophagy and apoptosis mediators in the different phases of liver diseases.

n.3 In figure 3 we reported the Western Blot analysis of eight pairs of HCC and the corresponding surrounding cirrhotic tissues. A, C and D show the protein expressions relative to patients n.1, 2, 3 and 4 while B and E the protein expressions relative to patients n. 5, 6, 7 and 8.

n. 4 In our study most tissues used for Western Blot and Real time PCR analysis were obtained from patient with HCC arising in HCV infected cirrhotic tissues. The paper by Takehara T et al does not report whether the HCC was in cirrhotic or not cirrhotic liver, any information regarding etiology; therefore this may in part explain the difference in Bcl-xL protein expression observed in the two studies. But I stress that our data are consistent with those reported by Daniel et al: decreased levels of BCI-xL in HCC compared with surrounding HCC tissues, even though the Authors have not observed any statistical significant difference in Bcl-xL between HCC and the correspondent surrounding liver tissue, while we did.
Point to point answer to Referee n 2

In the abstract the results regarding Bcl-2 expression are now included.

**Major Compulsory Revisions**

n 1. The correlation graph between Beclin 1 and Bad is now included in figure n.4: indeed a statistically significant correlation is present but the r value is higher for the correlation with Bcl-xl.

n 2. The different profile expression of Bcl-2 and Bcl-xl, redundant proteins, in the respect to cirrhosis and CH, can not be compared to previous studies in the literature. In our experience it looks like that the Bcl-2 overexpression characterizes only the late phases of the disease i.e. when HCC develops, while Bcl-xl expression begins in earlier phases of the disease itself. In the discussion section the different profile of Bcl-2 and Bcl-XL expression is elucidated.

n 3 In Figure 3 Bcl-xl protein levels were showed.

n 4 Figure 3 was substituted with a new graph reporting densitometric analysis of Western blot.

**Minor Essential Revisions**

In the abstract, background and discussion the statement “the main autophagic agent “ has been changed in “one of the main autophagic agents”

Minor concern not for publication:

In material and methods, the p53, p21 and BI-1 primers sequences reported Primers and Western Blot analysis sections were deleted.

The densitometric analysis of protein expression was showed in figure 3.