Author's response to reviews

Title: Abnormal expression and processing of the proprotein convertases PC1 and PC2 in human colorectal liver metastases.

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To the Editors of BMC Cancer

RE_Abnormal expression and processing of the proprotein convertases PC1 and PC2 in human colorectal liver metastases.

Thank you for the opportunity to revise the manuscript. As you will see there have been many changes, mainly involving the addition of protein data from 5 primary colon cancer specimens. The analysis in the primary colon cancers is not quantitative but qualitative, since the results are not homogeneous.

Responding the comments of Dr. Berreta the Figure 1C is representative of the PC1 immunoblots (pictures from 2 out of 14 patients) while the mean amount of protein PC1 in tumor is 2.5- fold than in unaffected or in normal liver (page 6). In the methods section, the statistical analysis is explained. Finally these results are corroborated from the immunohistochemistry Fig 4A/B/C.

The comments by Dr. Rosenbaum were very well received and thus we proceeded with analysis of PC1, PC2 and 7B2 protein expression and processing with immunoblot and immunohistochemistry in 5 primary colon cancers (Figure 7 and supplementary data). We did not perform a quantitative analysis in the protein results since they appeared not to be homogeneous.

The control gene used for the PCR analysis was GAPDH (page 5, par 3), and the annealing temperatures as well as the primers used are described in the methods section.

The statistical methods are also described in the methods section.

New pictures have been shot from unaffected liver and corroborate with the results.

The vertical axis in figure 2 has been corrected.

The comments regarding a potential neuroendocrine profile of colon cancer has been kept to a minimum, and the reference has been correctly referenced.

Thank you in advance

George N. Tzimas, FRCSC
Figure Legends for supplementary data

**Figure 1**: Photograph of liver resection specimen. Tumor (T) and unaffected (U) areas of the liver are indicated (arrows).

**Figure 2**: A. Positive control for immunohistochemistry with PC1 antibody. Light microscopy immunohistochemistry of mice brain (1, 2), using 100 X magnification. B. Negative control. C. Positive control for immunohistochemistry with PC2 antibody. Light microscopy immunohistochemistry of mice brain (1, 2), using 100 X magnification. D. Negative control. E. Positive control for immunohistochemistry with 7B2 antibody. Light microscopy immunohistochemistry of mice brain (3, 4) using 400 X magnification. F. Negative control.

**Figure 3**: A. Light microscopy PC1 immunohistochemistry of primary colon cancer using 400 X magnification. Note the positively stained tumor cells. B. Negative control for PC1. C. Light microscopy PC2 immunohistochemistry of primary colon cancer using 400 X magnification. Note the positively stained tumor cells. D. Negative control for PC2. E. Light microscopy 7B2 immunohistochemistry of primary colon cancer using 400 X magnification. Note the positively stained tumor cells. F. Negative control for 7B2.

References