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Relazione di ricerca CNR STM 2014

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Evaluation of UbcH10 expression during hepatocarcinogenesis.

Sincerely
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Evaluation of UbcH10 expression during hepatocarcinogenesis.

The main purpose of my stay in the laboratory of Prof. Terracciano during the fruition of the CNR STM 2014 fellowship was to analyze the levels of UbcH10 in hepatocellular carcinoma (HCC) samples. In agreement with Prof. Terracciano, we decided to analyze a tissue microarray (TMA) containing 221 HCC samples, most of them paired with the corresponding cirrhotic (precancerous lesion) or normal area.

Initially, I focused on the development of antigen-antibody reaction in order to perform an optimal staining of the TMA in object. To do this, I selected 10 whole sections corresponding to different human cancer tissues, including liver, and I proceeded to optimize the staining protocol by using different concentrations of an antibody able to specifically recognize the human UbcH10. With the support of immunohistochemistry laboratory technicians, I chose the best antibody concentration and then stained the TMA. After the staining procedures, the TMA was mounted with cover glass and was assessed by me together with Prof. Luigi Terracciano, an expert liver pathologist. In the specific, for each core corresponding to the tumor and normal liver tissue, we evaluated at the same time the percentage of cells expressing UbcH10 and the intensity of the expression. Then, the values obtained were inserted in the appropriate tables and particular associations were evaluated. In the figure below I show a representative staining of a neoplastic core and its paired corresponding normal core of the same patient included in the TMA (Figure 1).

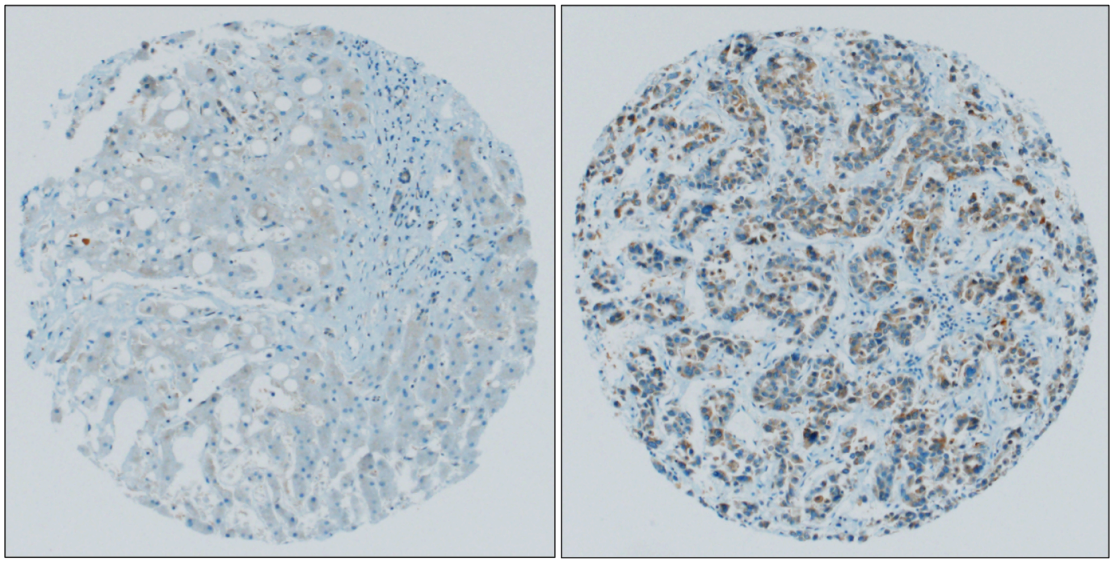

Figure 1. Representative pictures corresponding to the staining of UbcH10 in a normal liver (left) and a liver carcinoma (right) of a paired lesion included in the TMA.

In particular, we found that about 10% of HCC samples showed UbcH10 overexpression compared to the corresponding normal tissues, which never expressed UbcH10. This finding is quite interesting since these patients showing an abundant expression of UbcH10 had a worst prognosis as far as the overall survival is concerned. Additionally, in these tumor samples the overexpression of UbcH10 was negatively associated with the expression of E-cadherin, a known marker of differentiation. This result is very interesting since it indicates that the overexpression of UbcH10 might play a pivotal role in the neoplastic transformation of the hepatocytic cell because it promotes the loss of cellular differentiation, making it more aggressive with regards to its biological behavior. Furthermore, the result confirms the data reported in the literature regarding the overexpression of UbcH10 in other human carcinomas.

As far as cirrhosis is concerned, the TMA analysis shows that the overexpression of UbcH10 is not associated with the emergence of these latters in comparison to the normal liver tissue.

To investigate more thoroughly the possible role of UbcH10 during neoplastic progression of the hepatocytic cell starting from inflammation, I also performed an expression analysis by using real time PCR. To do this, under the supervision of Prof. Terracciano, I selected 30 fresh-frozen specimens from the tissue bank of the University of Basel, Pathology, including 8 normal livers, 6 steatosis, 6 steatohepatitis and finally 10 HCC. After selecting the most appropriate specimens, with the support of the technical staff of the immunohistochemistry laboratory, several fragments were obtained from each selected block, then, I proceeded to extract RNA by using an appropriate Qiagen kit. I checked the quality of RNA on agarose gel and then I made a real time PCR to assess the expression of UbcH10 in the series of selected samples. As internal control I used the 18S normalizer gene and all the samples were evaluated with respect to the normal liver tissues.

Also in this case, the data are very interesting since UbcH10 is abundantly expressed in 50% of liver carcinoma cases, but additionally it can be noted a slight increase of expression even in intermediate lesions, such as steatosis and steatohepatitis. Below is depicted the data obtained by real time PCR in which it can be observed the overexpression of UbcH10 in precancerous lesions and expecially in carcinoma cases (Figure 2).

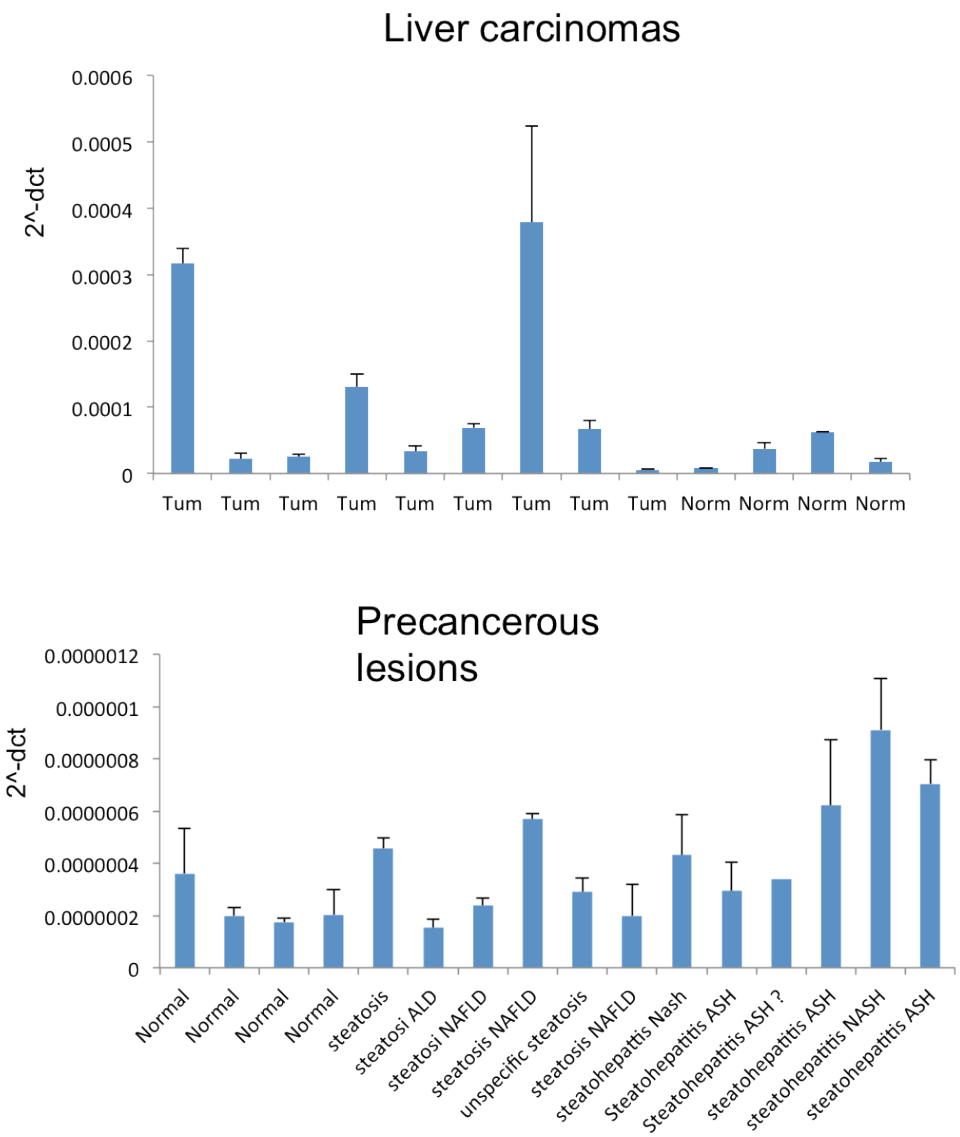

Figure 2. Evaluation of UbcH10 mRNA expression by real time PCR in liver precancerous lesions and carcinomas. Y-axis values are directly related to the expression of UbcH10. Norm, normal liver; Tum, liver carcinoma.

Therefore, through the use of real time PCR, a method much more sensitive if compared to immunohistochemistry, UbcH10 resulted partially overexpressed even in liver precancerous lesions, and this result confirms its involvement in tumor progression. Furthermore, the real time PCR confirmed that the deregulated expression of UbcH10 also occurs at the transcriptional level, and not only at the protein level.

In conclusion, thanks to the time spent in Basel University by taking advantage of the CNR STM 2014 fellowship, I was able to evaluate the expression of UbcH10 in HCC system confirming its overexpression at both protein and mRNA levels. Prof. Terracciano, head of my stay at the University of Basel during this period, is very enthusiastic about the results obtained in collaboration and hopes for a continuation of the collaboration between the University of Basel and the National Research Council, in the nearest future. I really appreciated this CNR opportunity and as I hope to continue this collaboration in future.