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Specificities of Hepatocellular Carcinoma Developed in Non Alcoholic Fatty Liver Disease in Absence of Cirrhosis Revealed by Tissue ¹H-NMR Spectroscopy



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1. BACKGROUND

Hepatocellular Carcinoma (HCC) accounts for the third cause of cancer mortality worldwide (1). HCC developed in Non Alcoholic Fatty Liver Disease (NAFLD) occurs in 40% of cases in the absence of cirrhosis and therefore may escape detection enabled by systematic screening of cirrhotic patients (2). To get insights into the biology of HCC in non-cirrhotic NAFLD and seek for putative cancer pathways, we performed metabolomics in HCC developed in cirrhosis and non-cirrhotic NAFLD.

2. MATERIALS & METHODS

Metabolomic was performed using ¹H-NMR Spectroscopy. The analysis included 28 pairs of HCC tissue and distant Non Tumoral Tissue (NTT) collected from patients undergoing hepatectomy. HCC was associated with cirrhosis (n=9), normal liver (n=6) or NAFLD (n=13). Multivariate Statistical Analysis of spectral data of aqueous and lipid tissues extracts are presented. The study was approved by the ethics committee.

3. RESULTS

I-Comparison of HCC Tissues versus Non Tumoral Tissues

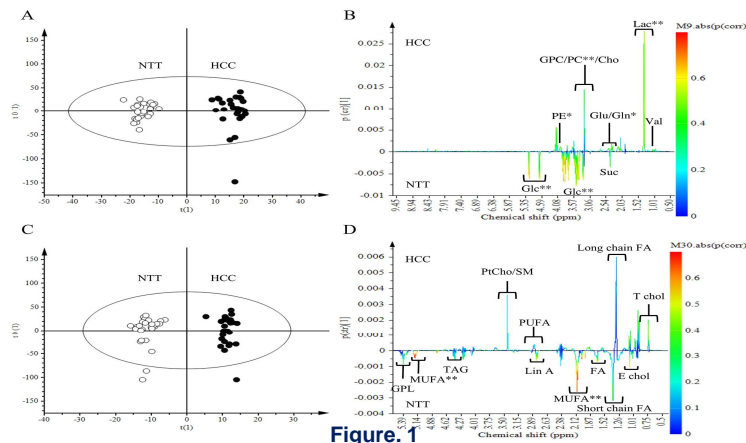


Figure 1

Tissue spectra of HCC and NTT groups were separated by OPLS-DA with aqueous extract data and lipid extract data (Fig.1 A and C). Multivariate analysis showed that HCC tissue is characterized by high level of lactate (Lac) ($p(\text{corr}) > 0.7$), phosphocholine (PC), phosphoethanolamine (PE), glutamine (Gln) ($p(\text{corr}) > 0.5$) and low level of glucose (Glc) and non-unsaturated fatty acids (MUFA) ($p(\text{corr}) > 0.7$) (Fig. 1 B and D).

III- Glutamine synthetase (GS) immunostaining

HCC-NAFLD was characterized by intense immunoreactivity of GS (Fig. 4 A and B). Gln quantification was correlated with GS staining, evaluated by a H-score (0-300) calculated by multiplying staining intensity (0, negative; 1, weak; 2, moderate; 3, strong) with the positively stained area (0-100%) (Fig. 4 C). OPLS-DA with spectra data shows a discrimination between HCC with a GS H-score ≥ 180 and HCC with a GS H-score < 180 . Fig. 4 D).

Signals contributing to the discrimination ($p(\text{corr}) > 0.50$), Gln (2.45 ppm) was found in HCC exhibiting a high GS staining whereas GSX (2.55 ppm) and lysine (2.96 ppm) were found in HCC with a weak GS staining (Fig. 4 E). Lys is a ceto-genic amino acid involved in beta-oxidation of fatty acids; this metabolite may explain the over-expression of β -HB found in HCC developed on cirrhosis.

4. CONCLUSION

This study provides evidence of metabolic specificities of HCC developed in non-cirrhotic NAFLD versus HCC developed in cirrhosis. These alterations could suggest activation of glutamine synthetase pathway in HCC-NAFLD and mitochondrial dysfunction in HCC-cirrhosis, that may be part of specific carcinogenic processes.

II- Comparison of HCC developed in cirrhosis versus HCC developed in NAFLD

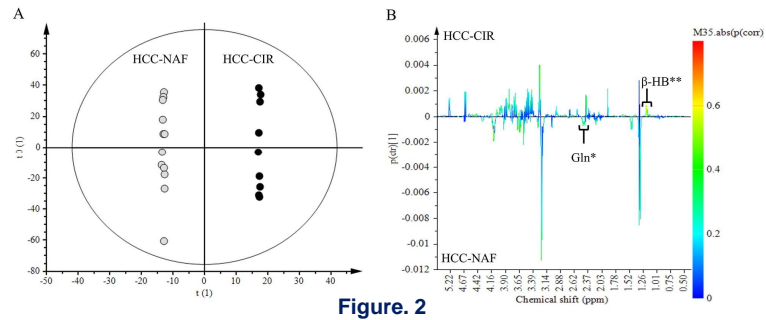


Figure 2

NMR spectra of aqueous extracts of HCC associated with non-cirrhotic NAFLD (n=13) were compared to those of HCC with cirrhosis (n=9). Among signals contributing to the discrimination, 2 metabolites were identified: β -hydroxybutyrate (β -HB) (1.18 ppm) and Glutamine (Gln) (2.45 ppm). β -HB ($p(\text{corr}) = 0.58$) was highly expressed in HCC-cirrhosis whereas Gln ($p(\text{corr}) = 0.45$) was highly expressed in HCC-NAFLD (Fig. 2B).

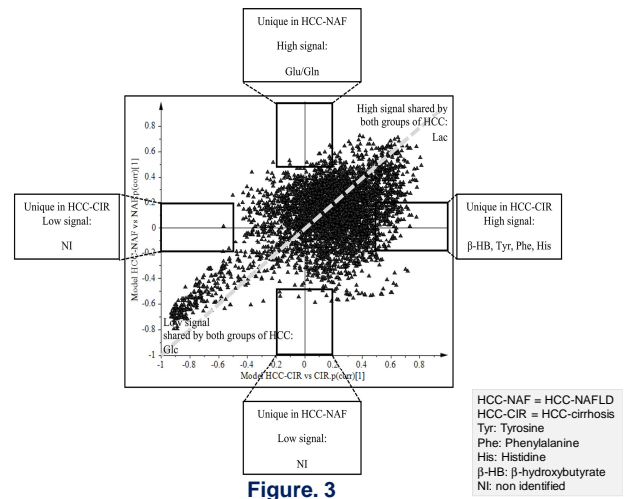


Figure 3

OPLS-DA statistical models of HCC-cirrhosis vs NTT (cirrhosis) and HCC-NAFLD vs NTT (NAFLD) were constructed before comparing them in shared and unique structure (SUS) plots. From SUS-plot (Fig. 3). HCC-Cirrhosis was characterized by high levels of β -HB, Tyr and Phe, whereas HCC-NAFLD was characterized by high levels of Gln/Glu.

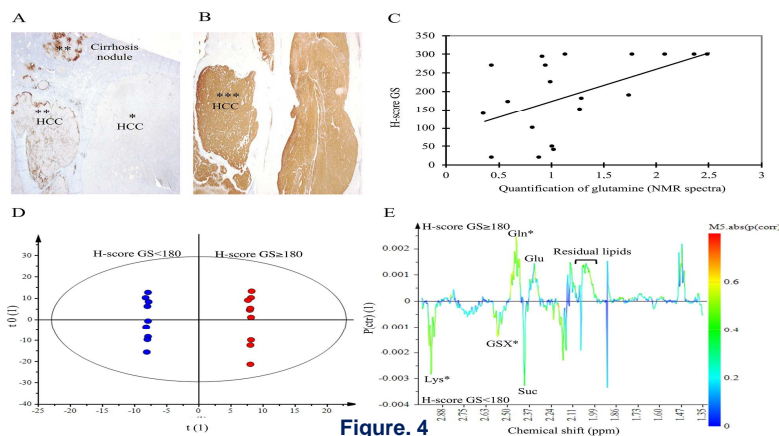


Figure 4