Longitudinal Metabolic Imaging of Hepatocellular Carcinoma in Transgenic Mouse Model Involving HBsAg and Aflatoxin B1 Risk Factors Identifies Altered Carnitine metabolism

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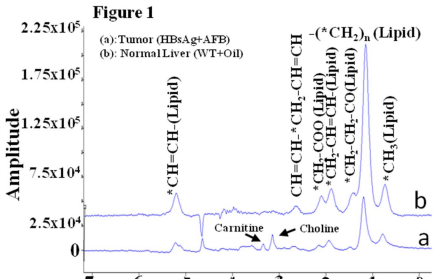
Introduction: Hepatocellular carcinoma (HCC) is one of the most deadly forms of the cancer in the world with poor prognosis and a 5-year survival of about 5 %[1]. Hepatitis B, hepatitis C and aflatoxin B1 (AFB1) exposure are the major known causes of HCC[2]. MR Image confirmation of a neoplasm usually occurs at later stages of tumor growth. In some cases of neoplasm which are beyond the imaging sensitivity limits requires confirmation by invasive biopsy techniques. Even before tumors are seen, there are crucial metabolic perturbations involving the changes in lipid composition[3]. Indentifying these early changes at pre-neoplastic stage of the tumor growth would increase the efficacy and therapeutic outcome. In the present study, to assess the independent risk factors of hepatitis B and aflatoxin exposure, we have used four groups (three HCC groups and one control group) of HCC mice models which closely mimic the human HCC. We investigated the early metabolic changes in HCC tumors including degree of unsaturation (DU) and apparent diffusion coefficient (ADC) in HCC and control groups.

Methods: All the *In-vivo* experiments were performed as per the approved IACUC protocol. There were four groups of animals used in this study. Group 1: HBsAg+AFB1 (n=10) is obtained by treating HBsAg (Hepatitis B surface Antigen as a transgene) expressed transgenic mouse model with AFB1. Group 2: WT +AFB1 (n=6) is obtained by treating WT type mouse treated with AFB1. Group 3: HBsAg + Oil (n=4) is obtained by treating HBsAg expressed transgenic mouse with oil. Group 4 : WT+Oil (n=4) is obtained by treating WT type mouse with oil. AFB1 and corn oil treatment was performed on the 7th day after the birth. These four groups of animal models were subjected to MRI and MRS study at different weeks of animal age before any visible tumor growth. All MR experiments were performed with a 7 T ClinScan MRI/MRS scanner (Bruker, Karlsruhe, Germany) equipped with a 72-mm volume resonator for RF transmission, in combination with a 20-mm surface receive-only coil. All experiments were respiratory gated to obviate the effects of liver motion. MRI experiments included gradient echo, spin echo; and multi-directional DTI (b = 0, 1000 s mm^{-2}) with 20 gradient directions over the entire liver. A volume-localized PRESS sequence was employed on normal liver, and the tumor lesions, with TR=4 s, TE=13 ms, number of transients Av=128, voxel size=2×2×2 mm^3, spectral width =3500 Hz, with 2048 complex points per free induction decay. The choline and carnitine concentrations were estimated from the MRS spectra using the unsuppressed water signal from the same location. Java based ImageJ plugin was developed for processing the DTI and ROI analysis. The apparent diffusion coefficient (ADC) was calculated using standard method[4].

Results and Discussion: Out of the four groups of animal's models, group (Gp) 1, 2 and 3 were HCC models with tumors. Group 4 served as a control for the rest of the animals. Figure 1 shows the representative spectra from HCC group HBsAg + AFB1 (Gp1) and control group WT + Oil (Gp 4). In the current work we have observed the carnitine (in addition to choline) in tumors of Gps 1, 2 and 3 HCC models whereas the carnitine was not observed in control model (Gp 4). Figure 2 shows the choline content of the tumors of Gps 1, 2, 3 and 4. The tumor choline content of Gps 1, 2 and 3 was significantly higher ($p < 0.05$) than control liver (Gp 4). Figure 3 shows the carnitine levels in Gp 1, 2 and 3. Carnitine was not observed in Gp 4. Carnitine content in the HCC tumors of Gp 1 was significantly higher ($p<0.05$) compared Gp 2 and Gp 3 tumors. Figure 4 shows the degree of un-saturation evaluated for all four groups. The DU of the tumor in all three HCC groups (Gp 2, 3 and 4) was significantly lower than normal liver with $p < 0.005$ (Gp 4). Figure 5 shows the tumor and normal liver ADC values for all four groups of animals. Tumor ADC value for Gp 1 is 0.738 x 10^{-3} mm^2/s, Gp 2 (0.779 x 10^{-3} mm^2/s) and for Gp 3 (0.807 x 10^{-3} mm^2/s). ADC values for all three groups of HCC tumors were significantly lower than normal liver with an ADC of 1.09 x 10^{-3} mm^2/s ($p < 0.005$).



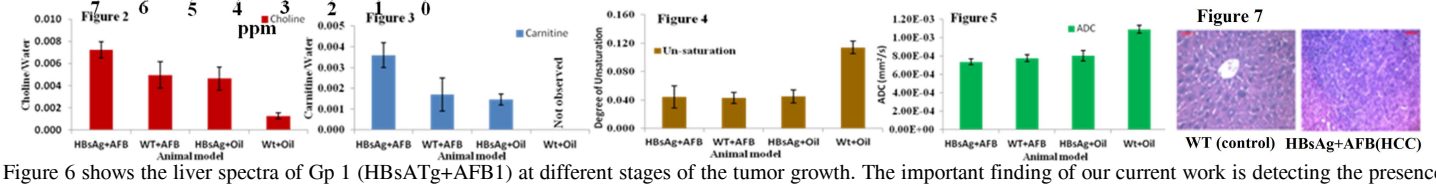
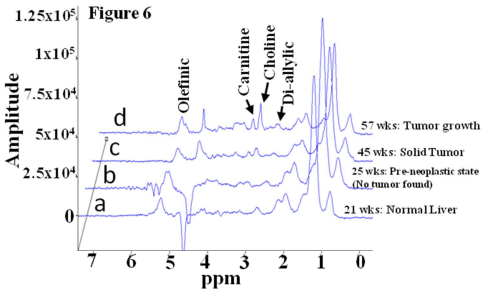


Figure 6 shows the liver spectra of Gp 1 (HBsATg+AFB1) at different stages of the tumor growth. The important finding of our current work is detecting the presence of carnitine even before the formation of neoplasm. In Figure 6, at 21 weeks (a) of age there were no tumors found and choline concentrations were similar to normal liver. Carnitine was not observed until 21 weeks. The ADC of normal liver at 21 weeks was 1.05 x 10^{-3} mm^2/s. At 25 weeks (b), although there were no visible tumors found in the liver the carnitine and choline were seen at low concentrations. Total amount of lipids were higher at this age. This is probably due to tissue demands for more lipid synthesis due to metabolic perturbations by tumor growth. ADC in this liver was lowered to 0.927x10^{-3} mm^2/s compared to the ADC at 21 weeks of age. At 45 weeks (c) of age, the tumor (tumor size: 7.5 x 8.5 mm^2) showed increase in concentrations of the carnitine and choline. The change in tumor physiology is indicated by significantly lower ADC of 0.766x10^{-3} mm^2/s compared to normal liver. At 57 (d) weeks of age the malignant transformation of the tumor has occurred with increased tumor size to 13 x 11 mm^2 and the ADC of the tumor was 0.774x10^{-3} mm^2/s. The concentrations of carnitine and choline were significantly higher than rest of the earlier weeks Decrease in peak areas of 2.7 ppm (di-allylic) and 5.2 ppm (olefinic) were noticed in the 57 weeks of tumor compared to 21 week normal liver. The degree of unsaturation (DU) at 57 weeks with tumors was significantly lower compared to the normal liver. Decrease in DU in tumors could indicate the alterations in the desaturase enzyme stearoyl CoA desaturase in the malignant tumors. Figure 7 shows the hematoxylin and eosin (H & E) sections of the control liver (WT) and HCC tumor (HBsAg+AFB1). Control mice showed signs of general degenerative changes only at 60 weeks of age, which were otherwise normal. On the other hand, liver tumors arising in the various groups of mice (Gp 1 to 3) are histologically similar, and showed altered morphology, and highly transformed compared to normal liver



Conclusions: Choline and carnitine metabolism was investigated in three HCC models. Carnitine was indentified in the liver even before the formation of neoplasm indicating the possibility of potential early marker even before tumors can be seen. Tumor carnitine and choline levels were at higher concentrations in HBsAg+AFB1 (Gp 1) HCC model compared to WT+AFB1 (Gp 2) and HBsAg+Oil (Gp 3) HCC model reflecting malignant transformation of the tumor. The degree of unsaturation in tumors was lower than normal liver indicating the altered lipid composition in the tumors. **References:** (1). El-Serag H.B. Hepatocellular carcinoma: an epidemiologic view, *J Clin. Gastroenterol*.,35,S72(2002);(2). Anthony P.P. Hepatocellular carcinoma: an overview, *Histopathology*,39,109,(2001);(3). J. Griffitts., et. al., *J. Lipid Res*.,50,611(2009);(4). Basser PJ et al. *J. Mag. Reson*.,111,209(1996).