Abstract: 12S

Taurodeoxycholic acid (TDCA) is a potential circulatory biomarker of NASH driven HCC

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Background: Hepatocellular carcinoma is one of the most common and rapidly rising cancers. Its swift and asymptomatic progression renders it the third leading cause of cancer-related deaths worldwide. Treatment efficacy depends on early disease diagnosis, which, to date, requires confirmation by an invasive and costly liver biopsy. Improving disease outcome, therefore, hinges on the discovery of new easy-to-assess biomarkers.

Methods: To this end, we have engineered the transgenic *MUP-uPA* mouse model of non-alcoholic steatohepatitis (NASH) driven HCC. When fed a Western diet (specifically a high-fat (HFD) or high-fructose (HFrD) diet), *MUP-uPA* mice develop all NASH hallmark characteristics including hepatocyte ballooning, steatosis, inflammation and fibrosis at ~24 wk of age, with only 50-60% of mice progressing towards HCC by 40 wk. It is this partial progression towards HCC that renders the *MUP-uPA* mouse model ideal for disease blood biomarker discovery.

Results: *MUP-uPA* mice were fed a HFD from 6 wk of age, blood samples were obtained at 24 wk, and mice were humanely killed at 40 wk. Untargeted metabolomics were performed on the 24 wk plasma samples and segregated into those that did or did not develop HCC at 40 wk. Despite the fact that all mice were phenotypically identical at 24 wk, taurodeoxycholic acid (TDCA) levels were ~10-fold higher (*P<0.01*) in the blood of those mice that subsequently developed HCC. We next confirmed these data in a cohort of human blood samples from healthy, non-alcoholic fatty liver disease (NAFLD) with cirrhosis, and HCC with fibrosis patients, and found TDCA levels to be significantly elevated in the HCC with fibrosis group only (P<0.01). TDCA is a secondary bile acid produced by the intestine and known to increases intracellular production of reactive oxygen (ROS) and nitrogen species (RNS) resulting in DNA damage, endoplasmic reticulum (ER) stress and increased inflammation.

Accordingly, we treated AML12 mouse hepatocytes with different doses of TDCA and found increased measures of ROS (hydrogen peroxide (H_2O_2) by AmplexÒ Red), inflammation (tumour necrosis factor (TNF)), ER stress (X-box protein 1 splicing), and DNA damage (P53). Finally, we developed a reliable, quantitative high-throughput assay to measure TDCA in liquid, allowing us to screen for this metabolite *in vivo*.

Conclusion: TDCA induces hepatic ROS, inflammation and ER stress *in vitro*, whilst being a circulatory biomarker of HCC development in the *MUP-uPA* mouse model.

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