Extended Abstract | Journal of Infectious Diseases and Treatments | 2020 Vol.6 No.2

Selected Probiotic Bacteria and Short Chain Fatty Acids Delay the Pathogenesis of Hepatitis B Virus-Associated Hepatocellular Carcinoma

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Chronic infection with hepatitis B virus (HBV) is associated with the development of progression of chronic liver disease (CLD) and the appearance of hepatocellular carcinoma (HCC). HCC is a prevalent cancer worldwide with few treatment options. Most of the treatments available are for patients with advanced cancer, and the results have been very modest because these are the patients most difficult to treat. A different way of thinking involves recognizing and reducing the risk factors that contribute to the pathogenesis of HCC. Given that HCC develops decades after HBV infection, and appears most often on the background of chronic inflammation, experiments were designed to test the hypothesis that selected probiotic bacteria and bacterial metabolites that mediate immunomodulation, by suppressing inflammation, could be used as a simple, readily available, and inexpensive means to target CLD so as to prevent or delay the appearance of HCC.

To test this hypothesis, hepatitis B x (HBx) transgenic mice, which develop CLD that culminates in the appearance of HCC, were treated with a mixture of probiotic bacteria (Synbiotic 2000TM). HBx is the oncoprotein of HBV that epigenetically alters host cell gene expression by activating multiple signaling pathways in the cytoplasm and stimulating histone deacetylase activity in the nucleus. In the transgenic mice, all develop hepatitis by 6 months of age, dysplasia by 9 months, and visible HCC by 12 months of age. Accordingly, mice were gavaged daily with Synbiotic 2000 or PBS spanning ages 6-9 months, and their livers were evaluated for dysplasia. In parallel, mice were gavaged daily from 9-12 months of age and evaluated for HCC. The results showed a significant reduction in serum alanine aminotransferase (ALT) values in treated compared to control mice evaluated at 9 ($P < 0.001$) and 12 months ($P < 0.001$) of age. Synbiotic 2000 treated mice showed decreased levels and tissue distribution of intrahepatic HBx compared to PBS fed transgenic mice based upon immunohistochemistry. This is not surprising, since the expression and activity of HBx is stimulated in an oxidative environment characteristic of CLD, and when inflammation is reduced (verified by H & E staining and decreased ALT), the levels of HBx should also diminish. In addition, there was a significant reduction in number and size of dysplastic nodules at 9 months of age ($P < 0.01$) and visible HCC nodules at 12 months of age ($P < 0.02$) in treated compared to control transgenic mice. Non-transgenic littermates fed Synbiotic 2000 or PBS had normal ALT and liver histology. Microarray analysis of selected immune and cancer associated mRNAs that are elevated in human HBV carriers with HCC were reduced in the livers of mice treated with Synbiotic 2000 compared to control mice. At 9 months of age, Synbiotic 2000 treated mice showed significantly decreased mRNA levels in one or more components of EGF, Notch, TGFβ, Akt, and Wnt signaling pathways, as well as decreased levels of pro-inflammatory TLR3 and IL-18 mRNAs (2.1-8.6-fold, average 2.8-fold). At 12 months of age, mRNA levels were strongly depressed in IFN, hedgehog, Notch, TGFβ, and those encoding multiple metalloproteases, as well as those mRNAs encoding innate immune signaling and selected cytokines (2.4-68-fold, average 16.6-fold). These results show that the probiotic bacteria in Synbiotic 2000 are capable of slowing down the pathogenesis of HCC. This implies that appropriate pro-bacterial species in the gut could down-regulate immune responses that are responsible for CLD progression onto dysplasia and HCC.

Since the bacteria used metabolize complex carbohydrates to short chain fatty acids (SCFAs), which are known to have anti-inflammatory properties in other systems, HBx transgenic mice were fed a combination of SCFAs alone (without Synbiotic 2000) in a parallel experiment. Livers from 12 mo. old mice were analyzed for changes in gene expression that are present at the age of tumor development by mass spectrometry-based proteomics. SCFA-fed mice had significantly fewer dysplastic nodules when livers were evaluated at 9 months ($P < 0.02$) and 12 months ($P < 0.05$) of age. The numbers of visible HCC nodules were significantly reduced in treated 12 month old mice compared to controls ($P < 0.001$). Among tumor bearing animals, SCFA treated mice had predominantly small (< 0.5 cm diameter) and medium (0.5-1 cm diameter) sized tumors instead of large tumor nodules (> 1 cm diameter) compared to PBS treated mice ($P < 0.001$). Pathway analysis of SCFA fed mice showed down-regulation of several signaling pathways altered by HBx in human CLD and HCC, including those involved in inflammation, Wnt, FGF, PI3K, EGF, IGF and Ras. Further work showed that treatment with SCFAs was associated with decreased activity of the Ras pathway, which is constitutively activated by HBx. Other proteins in the Ras signaling pathway were also strongly depressed by SCFA treatment. Many of these signaling pathways cross-talk with NF-κB, which are known to be activated by HBx and inhibited by SCFAs, suggesting that the down-regulation of NF-kB is a key to the mechanism whereby SCFA treatment

delays the onset of HCC. In vitro work showed that SCFAs reduced cell viability in HBx-transfected human HCC cell lines (Hep3B and Huh7) in a dose-dependent manner while the viability of primary human hepatocytes was unaffected, indicating that SCFA treatment has low or little toxicity to non-tumor cells. Thus, SCFAs delay the pathogenesis of HBV-associated HCC, possibly through inhibiting signaling via NF-κB, suggesting that they may be a simple, effective intervention against HBV associated CLD and HCC. This approach may also be useful delaying the development of other cancers that that arise on the background of chronic inflammation. Human clinical trials are now being planned to conclusively test this hypothesis.

Foot Note: This work is partly presented at 5th International Conference on Gastroenterology and Hepatology, November 23-24, 2020 at Abu Dhabi, UAE