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MICROBIOME–OBESITY–LIVER CANCER INTERACTION: SENESCENCE OF HEPATIC STELLATE CELLS AND BILE ACIDS PLAY NEW ROLES

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Yoshimoto S, Loo TM, Atarashi K, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature 2013;499:97–101.

The increased prevalence of obesity is a significant health concern in developed countries. Epidemiologic human studies and experiments using rodents show that obesity is associated with not only the manifestations of metabolic disease, such as diabetes and cardiovascular disease, but also with increased incidence of a number of cancers, including hepatocellular carcinoma (HCC). The incidence of HCC is increased in patients with hepatosteatosis accompanied with liver inflammation (referred to as nonalcoholic steatohepatitis) rather than patients with simple steatosis. Emerging evidences demonstrate that intestinal microbiome is among the key components in the progression of obesity and HCC (Nature 2006; 444:1027–1031; Cancer Cell 2012;21:504–516).

Yoshimoto et al demonstrated that senescence-associated secretory phenotype (SASP) of hepatic stellate cells (HSC) induced by a bacterial byproduct, deoxycholic acid (DCA), promotes obesity-associated HCC progression. The study initiated to confirm that chemical toxin-induced Ras mutation causes HCC only in obese mice fed a high-fat diet. This phenotype was reproduced in genetically obese *ob/ob* mice. This study found that cellular senescence markers such as *p21^{Waf1/Cip1}* and *p16^{INK4a}*, as well as DNA damage markers (53BP1 and γ -H2AX), were up-regulated in HCC lesions, particularly in HSC. These HSC produced SASP cytokines, such as interleukin (IL)-6, GRO α , and CXCL9, in which proliferation was inhibited, demonstrating that HSC in HCC lesions has become senescent. Although HSC do not produce IL-1 α , a SASP contributor, HSC induced the expression of inflammasome components and IL-1 β . In IL-1 $\beta^{-/-}$ mice, a high-fat diet induced hepatosteatosis and HSC senescence, as demonstrated by the expression of 53BP1 and p21; however, the production of SASP cytokines and HCC formation were suppressed, suggesting that SASP is essential for obesity-associated HCC development.

The study then highlighted the contribution of intestinal microbiome to HCC development in mice. Gut sterilization by oral administration of antibiotics cocktail or vancomycin that targets Gram-positive bacteria significantly suppressed SASP of HSC and growth of HCC. They also found that serum levels of DCA and the composition of *Clostridium* cluster XI and XIVa that convert primary bile acids to DCA were increased in obese mice, but the DCA content was decreased by antibiotic treatment. Because DCA produces reactive

oxygen species that cause DNA damage and SASP, the researchers then focused on the crucial role of DCA in obesity-associated HCC growth. High-fat diet–driven HCC growth was dramatically suppressed by lowering DCA levels by administrations of difructose anhydride III, which inhibits 7 α -dehydroxylation activity or by ursodeoxycholic acid, which promotes bile acid secretion. Conversely, oral DCA feeding enhanced HCC development along with HSC senescence and SASP in obese mice. Finally, the study confirmed the presence of senescent HSC and SASP in HCC developed in nonalcoholic steatohepatitis patients.

Comment

Obesity and excessive nutrient consumption can promote HCC development, which has been shown in epidemiologic human studies as well as animal experiments (Hepatology 2010;51:1820–1832; Cell 2010;140:197–208). Obesity is known as systemic inflammatory disease. Proinflammatory cytokines, such as tumor necrosis factor- α and IL-6 released from adipose tissues and liver macrophages may contribute to the progression of nonalcoholic fatty liver disease. Reactive oxygen species produced during fatty acid β -oxidation or by stimulation with saturated free fatty acids also participate in nonalcoholic fatty liver disease development (Annu Rev Pathol 2010;5:145–171). In addition, overgrowth and changes in the composition of intestinal microbiome are associated with obesity (Hepatology 2013;58:120–127). The study done by Yoshimoto et al demonstrates that DCA, a metabolite of intestinal microbiome, causes DNA damage that induces SASP in HSC, resulting in the promotion of obesity-associated HCC.

One of their unique hypotheses is that HSC senescence and SASP-related cytokines promote obesity-associated HCC. Cellular senescence is a protective mechanism against tumorigenesis (Nat Rev Cancer 2009;9:81–94). Telomerase shortening, DNA damage, and oncogene activation, such as *Kras* and *Braf*, participate in the induction of cellular senescence. Senescence-induced cell-cycle arrest and apoptosis prevent malignant transformation and malignant cell growth. Yoshimoto et al found a number of p21-expressing senescent cells in HCC lesions, and determined that those cells are mainly HSC. HSC exhibit proinflammatory phenotype rather than fibrogenic phenotype during senescence (Hepatology 2003;37:653–664). In this context, proinflammatory cytokines, such as IL-6, GRO α , and CXCL9, produced from senescent HSC promote obesity-associated HCC. The researchers concluded that the responsible cells that produce SASP cytokines are senescent HSC, but not Kupffer cells, which are the major proinflammatory cytokine-producing cells in the liver. This hypothesis contradicts previous studies reported by Karin et al, demonstrating that Kupffer cells are the source of proinflammatory cytokines driving HCC development (Science 2007;317:212–214; Cell 2010;140:197–208). Alternatively, Schwabe et al have reported that intestinal microbiome-dependent TLR4-dependent profibrogenic response and epiregulin production in HSC contribute to fibrosis-associated HCC progression (Cancer Cell 2012;21: 504–516). Intriguingly, the study done by Lujambio et al demonstrates another hypothesis for HCC development associated with HSC senescence (Cell 2013;153:449–460). p53 is a tumor suppressor and senescence mediator associated with cell-cycle arrest and apoptosis. Lujambio et al generated HSC-specific p53-deficient mice in which HSC do not undergo senescence. Mice with p53-

deficient HSC produced more liver cirrhosis and accelerated HCC growth, indicating that HSC senescence suppresses liver fibrosis and HCC development. Moreover, the study found that p53-null HSC changed macrophage polarization to M2 phenotype. This hypothesis is contradictory to the hypothesis proposed by Yoshimoto et al.

Yoshimoto et al also demonstrate an intriguing observation that fibrosis does not contribute to obesity-associated HCC promotion. Although HSP47 is a specific molecular chaperone for assembly of collagen fibers, knocking down HSP47 in vivo reduces HCC growth. This suggests that HSP47 may have functions other than collagen assembly as a molecular chaperon, such as induction of HSC apoptosis (Nat Biotechnol 2008;26:431–442). Further investigations, such as using HSC-specific deletion technology, are required to answer the question whether HSC are the responsible cell types to promote the development of obesity-associated HCC.

Another key discovery of this paper is the contribution of intestinal microbiome to promotion of obesity-associated HCC. Although the changes in the composition of intestinal microbiota in obese population have been shown (Nature 2006;444:1027–1031), the mechanism is still unclear. Schwabe et al showed that TLR4 ligand, such as lipopolysaccharide, released from intestinal microbiome is a crucial component to promote fibrosis-associated HCC through HSC activation (Cancer Cell 2012;21:504–516). However, Yoshimoto's study demonstrated that TLR4 deficiency does not protect mice from obesity-associated HCC. Instead, they found that microbial byproduct DCA was generated as secondary bile acids through *Clostridium* in obese mice. DCA is known to induce reactive oxygen species production and causes genotoxic DNA damage as a potential carcinogen. DCA content is increased in feces from humans who consumed a high-fat, Western-style diet. Although the authors found that DCA causes HSC senescence, the direct effect of DCA on hepatocytes to malignant transformation has yet to be noted. In addition, the mechanism of DCA-induced HSC senescence is unclear. Bile acid receptors FXR and TGR5 may contribute to DCA-induced HSC senescence. Although the proposed role of HSC senescence on hepatocarcinogenesis is controversial with other studies, this paper undoubtedly demonstrates a novel pathophysiologic link between gram-positive bacteria-dependent DCA generation and obesity-associated HCC growth. This discovery provides the new idea to develop effective therapies and prevention targeting HSC senescence, bile acids and intestinal microbiome for nonalcoholic fatty liver disease-mediated HCC.