Introduction

Over the past several decades, obesity has become more prevalent in most developed countries and is increasingly recognized as a major risk factor for several common types of cancer (1–3). Although weight loss by exercise and/or dietary control ameliorates obesity-induced metabolic syndromes, the worldwide obesity epidemic has shown no signs of abating (3). Therefore, more effective methods are needed to prevent obesity-associated cancer development. Toward this purpose, a better understanding of the mechanisms underlying obesity-associated cancer is urgently required.

Cellular senescence is the state of irreversible cell-cycle arrest. It is primarily induced by persistent DNA damage responses (DDR) triggered by a variety of potentially oncogenic stimuli, such as telomere erosion, oxidative stress, or activation of certain oncogenes (4–6). Thus, cellular senescence has long been considered to act as an important tumor suppression mechanism (7, 8). However, in addition to stable cell-cycle arrest, it has recently become apparent that senescent cells also develop other characteristic features, such as widespread changes in chromatin organization and gene expression (9). A particularly important biological phenomenon is the increased expression of genes encoding a series of secreted proteins, such as inflammatory cytokines, chemokines, and matrix remodeling factors, in senescent cells. This newly identified senescent phenotype, termed the senescence-associated secretory phenotype (SASP; ref. 10) or the senescence messaging secretome (SMS; ref. 5), hereafter referred to as SASP, is induced by DDRs (11, 12) and contributes positively or negatively to cancer development, depending on the biologic context (13–15). Because some of the SASP factors, such as interleukin (IL)-6 and plasminogen activator inhibitor-1, are reportedly associated with increased cancer risk in obesity (1, 16), we explored the possibility that SASP may contribute positively to obesity-associated cancer development using the obese mouse as a model system.

Obesity Promotes Cellular Senescence and Liver Cancer Development

In contrast to studies in humans, we were unable to detect any statistically significant difference in cancer development between obese mice fed a high-fat diet (HFD) and lean mice fed a normal diet. However, because laboratory mice do not smoke or drink alcohol and are kept in a very clean environment, such as a specific pathogen-free facility, we speculated that a certain level of oncogenic stimuli might be needed to reveal the impact of obesity on cancer development, especially in wild-type mice maintained in a specific pathogen-free environment. Hence, we decided to treat the mice with 7,12-dimethylbenz(a)anthracene (DMBA, a chemical carcinogen that causes an oncogenic ras mutation) at the neonatal stage, because this protocol is known to generate a variety of tumors throughout the body (17). We also took advantage of the p21-luc mouse strain, in which the expression of the p21Waf1/Cip1 gene (a critical inducer of cellular senescence) can be monitored noninvasively by a bioluminescence imaging technique in living mice (18). Using male p21-luc mice, in conjunction with DMBA treatment at the neonatal stage followed by feeding an HFD or normal diet for 30 weeks, we analyzed whether obesity promotes tumorigenesis in mice...
and if cellular senescence is involved in obesity-associated cancer. Intriguingly, we found that obese mice, but not lean mice, developed hepatocellular carcinoma with upregulated p21Waf1/Cip1 gene expression in hepatic stellate cells (HSC), adjacent to the hepatocellular carcinoma nodules detected by bioluminescent signals (19).

Although we were unable to detect two widely used cellular senescence markers, senescence-associated β-galactosidase (SA-β-gal) activity and senescence-associated heterochromatin foci (SAHF; refs. 20 and 21), in the HSCs expressing p21Waf1/Cip1, these HSCs displayed many other relevant features of cellular senescence, such as accumulation of DNA damage foci, elevation of reactive oxygen species levels, upregulation of p16Ink4a expression, cell-cycle arrest, and induced expression of a series of SASP factors (19). Moreover, accumulating evidence has indicated that SA-β-gal and SAHF are not required for cellular senescence (22–25). These findings, together with our observations of similar results in genetically obese (Lepob/ob) mice, suggested that obesity provokes senescence-like features in HSCs and promotes tumorigenesis in hepatocytes adjacent to these HSCs (19).

The Roles of SASP in Hepatocellular Carcinoma Development in Obese Mice

To clarify the roles of the senescence-like features in the HSCs, we repeated the same experiments using mice lacking IL-1β, a critical inducer of SASP (26, 27). Interestingly, although the degrees of DNA damage and cell-cycle arrest in HSCs were unchanged, significant reductions of SASP in HSCs and hepatocellular carcinoma development were observed, implying that SASP plays crucial roles in hepatocellular carcinoma development in obese mice (19). To obtain further support for this idea, we attempted to generate conditional knockout mice lacking IL-1β only in HSCs. However, there are currently no reports of genes expressed only in HSCs. Moreover, quiescent HSCs are known to be capable of functioning as multipotent progenitors and producing hepatocytes in adult livers (28), thus making it difficult to generate HSC-specific knockout mice. Therefore, we tried an alternative approach, the siRNA-mediated depletion of HSCs (29), and obtained the same results (19), indicating that senescent HSCs promote hepatocellular carcinoma development through SASP in obese mice. Importantly, however, a recent report from Lowe’s group has indicated that senescent HSCs suppress, rather than promote, hepatocellular carcinoma development through SASP in mice treated with diethyl nitrosamine (DEN) plus carbon tetrachloride (CCL4) (30). Thus, it is possible that these seemingly disparate results may reflect, at least in part, the status of the p53 gene in hepatocytes, although there are many other reconciliations, including gross differences in the models used (obesity vs. chemical liver injury) linked to likely differences in spatial and temporal activation of SASP, qualitative and quantitative composition of the SASP.

An Obesity-Induced Gut Microbial Metabolite Promotes Hepatocellular Carcinoma Development

How does obesity provoke the senescence-like features in HSCs? It has recently become apparent that alterations of the gut microbiota are associated with obesity in both humans and mice (30, 31). Furthermore, the activation of toll-like receptor 4 by lipopolysaccharide (LPS) from gut Gram-negative bacteria has been shown to promote hepatocellular carcinoma development in mice treated with DEN plus CCL4 (32). We explored this point further and found that altering the gut microbiota in obese mice by antibiotic treatment reduced the abundance of senescent HSCs and hepatocellular carcinoma development (19). Similar results were also observed in germ-free mice treated with DMBA at the neonatal stage followed by HFD feeding (Hara Laboratory, unpublished results), indicating that the obesity-induced alteration of gut microbiota is likely to provoke the senescence-like features in HSCs. Because we were unable to determine the role of LPS in our experimental setting and a treatment with vancomycin, an antibiotic that preferentially targets Gram-positive bacteria, alone was sufficient to block the appearance of senescent HSCs and hepatocellular carcinoma development (19), we hypothesized that Gram-positive bacteria may cause DNA damage in HSCs in obese mice. Indeed, a meta 16S rRNA gene-sequencing analysis revealed that the percentage of gut Gram-positive bacteria was strikingly increased by feeding an HFD to mice (19). Together, these results led us to speculate that the obesity-associated increase of gut Gram-positive bacteria may promote hepatocellular carcinoma development by provoking SASP in HSCs, presumably through the enterohepatic circulation of gut bacterial metabolites or toxins.

We then examined the serum via liquid chromatography/mass spectrometry and found that the level of deoxycholic acid (DCA), a secondary bile acid produced solely by gut bacteria such as Clostridium clusters XI and XIVa (vancomycin-sensitive Gram-positive bacteria), was substantially increased by the HFD feeding and was reduced by the antibiotic treatment (19). DFAIII-mediated suppression of the 7α-dehydroxylation of primary bile acids, the metabolic pathway for DCA production, or UDCA-induced stimulation of bile acid secretion reduced hepatocellular carcinoma development and markedly decreased the number of senescent HSCs in obese mice treated with DMBA at the neonatal stage (19). Conversely, prolonged treatment with DCA promoted hepatocellular carcinoma development in lean mice treated with DMBA at the neonatal stage (Hara Laboratory, unpublished results; ref. 19), consistent with previous reports that DCA has the potential to cause DNA damage and enhance liver carcinogenesis in rodents (33, 34).

Notably, an operational taxonomic unit (OTU)-based bacterial diversity analysis, in conjunction with a quantitative PCR analysis, revealed that the population of Clostridium cluster XI was strikingly increased in obese mice (19). Interestingly, a
Phylogenetic analysis of the bacterial OTUs revealed that Clostridium cluster XI is composed of a single bacterial taxon (OUT-1105) close to the DCA-producing strain Clostridium sordellii (35). Thus, although other bacteria may also participate, the simplest explanation for our data is that OUT-1105 contributes to an increase in the DCA level, at least to some extent, in obese mice.

Relevance to Human Disease

Finally, we asked whether our observations could be applied to humans. Note that at least four studies have found that obesity increases (by 1.5- to 4-fold) the risk of liver cancer (36–39). Moreover, in the setting of chronic hepatitis B or C infection, coexisting obesity has been shown to increase the risk for hepatocellular carcinoma by more than 100-fold (40). It should also be noted that obesity is a major cause of nonalcoholic steatohepatitis (NASH) and NASH is a risk factor for liver cancer (41, 42). Taken together, although the magnitude of the observed relative risk from existing studies is not consistent, it is clear that obesity increases the risk of liver cancer. These reports, together with the observations that the relative proportion of Firmicutes (Gram-positive bacteria) in gut microbiota increases in obesity, suggest a potential role for gut microbiota in the development of obesity-related liver cancer.

Figure 1. Obesity-induced hepatocellular carcinoma (HCC) development via senescence secretome. A, bile acid–transforming reaction in the intestinal tract. B, model for obesity-induced hepatocellular carcinoma development. Obesity induces alteration of gut microbiota, thereby causing promotion of DCA production in the intestinal tract. Elevated levels of DCA provoke SASP in HSCs presumably through enterohepatic circulation, which, in turn, secretes various inflammatory and tumor-promoting factors in liver.
microbiota is reportedly increased in obese people (30) and high fat consumption causes higher fecal DCA concentrations in healthy volunteers (43–44), prompted us to examine whether SASP is also associated with human obesity-induced liver cancers. Indeed, signs of cellular senescence and SASP were observed in the HSCs without serious fibrosis in the area of hepatocellular carcinoma arising in patients with NASH (19). These results are in good agreement with our murine data (19) and are consistent with recent reports showing that a certain percentage of NASH-associated hepatocellular carcinoma arises from the non-cirrhotic liver (42) and HSCs exhibit proinflammatory phenotype rather than fibrogenic phenotype during cellular senescence (45). Although we do not have a definitive answer yet, these findings, in conjunction with published reports (16, 30, 34, 43–46), strongly suggest that DCA-induced senescent HSCs may contribute to at least certain aspects of obesity-associated hepatocellular carcinoma development in human as well (see Fig. 1).

Remaining Issues and Directions for Future Research

Several issues remain to be resolved. For example, although many of the DCA–SASP axis perturbations, for example, the IL-1β knockout, antibiotics treatment, and lower DCA levels, significantly prevent hepatocellular carcinoma development in obese mice, residual hepatocellular carcinomas were still observed with these perturbations (19). Furthermore, DCA-feeding alone was insufficient to enhance hepatocellular carcinoma development in lean mice treated with DMBA at the neonatal stage until at least 30 weeks, although prolonged (55 weeks) DCA-feeding enhanced hepatocellular carcinoma development (Hara Laboratory, unpublished results). It is therefore tempting to speculate that one or more additional factor(s) associated with obesity may exist to promote obesity-associated hepatocellular carcinoma development. We are currently answering this question by identifying additional obesity-associated cancer-promoting metabolite through proteomics and metabolomics approaches.

It is also unclear why DCA causes irreparable DNA damage in HSCs, but not in other liver cells. Moreover, does DCA also affect cancers in other organs? It should be noted that a slight but substantial increase in lung cancer development was observed in obese mice treated with DMBA at the neonatal stage (19), and DCA has been shown to enhance colon carcinogenesis (47). It is therefore possible that DCA may also affect cancers in other organs depending on the biologic context. Along the same line, it remains unclear why the population of DCA-producing gut bacteria is increased in obese mice. However, because we have obtained similar results using Lepob/ob mice fed a normal diet, as compared with the results seen in obese mice fed an HFD (19), it is most likely that obesity, but not the HFD, increases the population of DCA-producing gut bacteria. We are currently investigating the mechanism underlying the obesity-associated increase of DCA-producing gut bacteria.

Finally, we need to determine whether the population of DCA-producing gut bacteria is increased by obesity in humans, and, if so, how this occurs. To address these issues, we are now collecting clinical samples to determine whether the levels of DCA and/or DCA-producing bacteria are higher in obese individuals than in nonobese individuals. If our results obtained from the mouse model translate to humans, then it may be possible to develop methods to predict obesity-associated cancer risk in the general population, such as by measuring the levels of DCA and/or DCA-producing bacteria in fecal samples. We are also interested in the possible benefits of treatments with prebiotics and/or probiotics in the prevention of DCA-producing gut bacterial growth. We hope that extensive research in this field will lead to the development of new strategies for cancer prevention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


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