Special Issue (Mini Review) Senescence and Gut microbiota
Inflammation and Regeneration Vol.35 No.3 May 2015

Special Issue: Interaction between gut microbiota and host immune cells

Mini Review

Cellular senescence and liver cancer: a gut microbial connection

Shin Yoshimoto[1,2,3], Tze Mun Loo[1,4] and Eiji Hara[1,2,*]

[1)]The Cancer Institute, Japanese Foundation for Cancer Research (JFCR), Koto-ku, Tokyo, Japan

[2)]CREST, Japan Science and Technology Agency (JST), Kawaguchi, Saitama, Japan

[3)]Present address: LSI Medience Corporation, Chiyoda-ku, Tokyo, Japan

[4)]Present address: Department of Applied Biological Science, Tokyo University of Science, Noda, Chiba, Japan

Due to over-nutrition and lack of exercise, the number of overweight and obese people in the world had surged in the past three decades, and is becoming one of the most serious public health problems worldwide. Extensive epidemiological studies reveal that obesity has major impacts on both the type and incidence of cancers. However, the exact molecular mechanisms underlying obesity-associated cancer are not fully understood yet. Using mouse models, we have recently found that dietary or genetic obesity provokes alterations of gut microbiota profile, thereby increasing the levels of deoxycholic acid (DCA), a gut microbial metabolite produced solely by the 7α-dehydroxylation of primary bile acids. The enterohepatic circulation of DCA increases the levels of DCA in liver and provokes DNA damage-induced cellular senescence in hepatic stellate cells (HSCs), which in turn, secretes various inflammatory and tumor promoting factors in the liver, thus facilitating hepatocellular carcinoma (HCC) development in obese mice. Notably, signs of cellular senescence and associated secretory phenotypes were also observed in the HSCs in the area of HCC arising in patients with non-alcoholic steatohepatitis (NASH) without cirrhosis, implying that a similar pathway is likely to contribute to at least certain aspects of obesity-associated HCC development in humans as well. In this review, we will provide an overview of our recent work and discuss the next steps, toward potential clinical applications of our findings.

Rec.2/4/2015, Acc.2/19/2015, pp106-113

*Correspondence should be addressed to:
Eiji Hara, Division of Cancer Biology, The Cancer Institute, Japanese Foundation for Cancer Research, 3-8-31 Ariake, Koto-ku, Tokyo 135-8550, Japan. E-mail: eiji.hara@jfcr.or.jp

Key words obesity, DNA damage response, cellular senescence, gut microbiota, liver cancer

Introduction

Over the past decades, human life expectancy has increased dramatically and people are now living longer, especially in developed countries. For example, average lifespan of most developed countries is around double compared with 100 years ago[1]. Ironically, however, extended lifespan has resulted in a startling rise in the incidence of age-related illnesses, such as cancer[2]. For instance, in Japan, now half the population will develop cancer in their lifetime and one-third of the population die of cancer. Thus, to have a meaningful impact on the extended lifespan, there is an urgent need for increased understanding of the molecular mechanisms underlying increased incidence of cancer in modern world.

Due to over-nutrition and lack of exercise, the number of overweight (defined as a body-mass index [BMI] of 25 to 29.9 kg/m^2) and obese (BMI of 30 kg/m^2 or greater) people in the world had surged in the past three decades. Multiple epidemiological studies have revealed that excess bodyweight is a major risk factor for not only diabetes and cardiovascular diseases but also cancer[3, 4]. Although weight loss by exercise and/or dietary control ameliorates obesity-induced metabolic syndromes, the worldwide obesity epidemic has shown no signs of abating[5, 6]. Therefore, more effective methods are required to prevent obesity-associated cancer development. Towards this purpose, better understanding of the mechanisms underlying obesity-associated cancer is urgently required.

Cellular senescence is a process occurring in normal cells in response to telomere erosion or oncogene activation, acting through checkpoint activation and stable cell cycle arrest as a barrier to tumorigenesis[7-13]. Recent studies, however, reveal that senescent cells also develop a secretory profile composed mainly of inflammatory cytokines, chemokines and proteases, a typical signature termed the senescence-associated secretory phenotype (SASP)[14, 15] or the senescence messaging secretome (SMS)[16], hereafter referred to as SASP. Some of the SASP factors display cell-autonomous activities to reinforce senescence cell cycle arrest[17,18] and/or promote tumor clearance[19-21]. Other SASP factors exhibit cell-nonautonomous functions associated with inflammation and tumorigenesis promotion[14], indicating that SASP contributes positively and negatively to cancer development, depending on the biological context[15]. Since some of the SASP factors, such as IL-6 and PAI-1, are known to increase cancer risk in obesity[4, 22], we have set up the system to explore the possibility that SASP may contribute to obesity-associated cancer development using wild-type C57BL/6 mice.

Dietary or genetic obesity induces cellular senescence in liver

In contrast to human epidemiological studies, 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 (ND). However, because laboratory mice do not smoke or drink alcohol and are kept in a very clean environment, such as a specific pathogen free (SPF) 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 SPF environment. Thus, we decided to treat the mice with DMBA (7,12-dimethyl benz(a)anthracene, a chemical carcinogen that causes an oncogenic ras mutation) at the neonatal stage, since this protocol is known to generate a variety of tumors throughout the body[23]. We also took advantage of the p21-*p*-luc mouse strain, in which the expression of the *p21$^{Waf1/Cip1}$* gene (a critical inducer of cellular senescence) can be monitored noninvasively by a bioluminescence imaging (BLI) technique in living mice[24]. Using male p21-*p*-luc mice, in conjunction with DMBA treatment at the neonatal stage followed by feeding a HFD or ND 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 (HCC) (see Fig. 1) with up-regulated *p21$^{Waf1/Cip1}$* gene expression in hepatic stellate cells (HSCs), adjacent to the HCC nodules detected by bioluminescent signals[25].

Although we were unable to detect two widely used cellular senescence markers, senescence-associated β-galactosidase (SA-β-gal) activity and senescence-associated heterochromatic foci (SAHF)[26, 27], in the HSCs expressing p21$^{Waf1/Cip1}$, these HSCs displayed many other relevant features of cellular senescence, such as accumulation of DNA damage foci, elevation of reactive oxygen species (ROS) levels, up-regulation of p16^{INK4a} expression, cell cycle arrest and induced expression of a series of SASP factors[25]. Moreover, accumulating evidence has indicated that SA-β-gal and SAHF are not always associated with cellular senescence[28-32]. These findings, together with our observations of similar results in genetically obese (*Lep$^{ob/ob}$*) mice, suggested that obesity provokes senescence-like

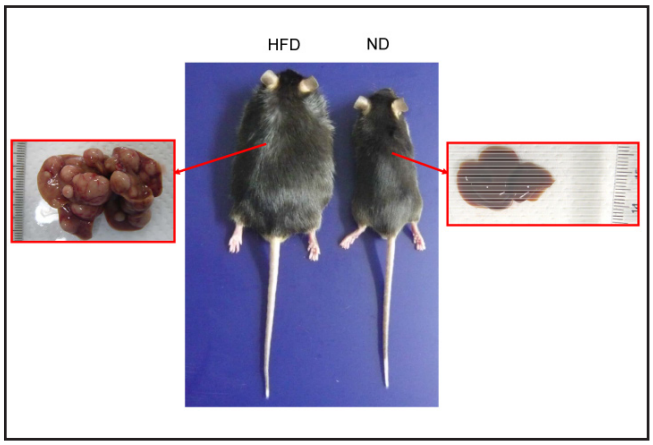

Fig. 1 Liver cancer in obese mice

Neonatal C57BL/6 mice were treated with DMBA, and then fed either HFD (left) or ND (right) for 30 weeks. These mice were euthanized and livers were rapidly removed and placed in culture dishes. Macroscopic photographs of livers are shown.

features in HSCs and promotes tumorigenesis in hepatocytes adjacent to these HSCs[25].

Senescent HSCs promotes HCC development through SASP 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[33, 34]. Interestingly, although the degrees of DNA damage and cell cycle arrest in HSCs were unchanged, significant reductions of SASP in HSCs and HCC development were observed, implying that SASP plays crucial roles in HCC development in obese mice[25]. 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 multi-potent progenitors and producing hepatocytes in adult livers[35], thus making it difficult to generate HSC-specific knockout mice. Therefore, we tried an alternative approach, the siRNA-mediated depletion of HSCs[36], and obtained the same results[25], indicating that senescent HSCs promote HCC development through SASP in obese mice. This is somewhat consistent with a recent report showing that colibactin, a bacterial genotoxin, promotes colon cancer development by inducing SASP in an AOM/DSS (azoxymethane/ dextran sodium sulphate) colon cancer mouse model[37]. Notably, however, a recent report from Lowe's group has indicated that senescent HSCs suppress, rather than promote, HCC development through SASP in mice treated with DEN (diethyl nitrosamine) plus carbon tetrachloride (CCl_4)[38]. It should be noted that the HCC obtained in our mouse model possessed a loss of function mutation in the p53 gene (Hara's lab, unpublished results), in contrast to the HCC arising in mice treated with DEN plus CCl_4[38]. Moreover, it has recently been shown that the senescence-associated inflammatory response suppresses or promotes tumorigenesis, depending on the p53 gene status[39]. 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 employed (obesity vs chemical liver injury) linked to likely differences in spatial and temporal activation of SASP, qualitative and quantitative composition of the SASP.

DCA, an obesity associated gut microbial metabolite provokes cellular senescence in HSCs

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[40, 41]. Furthermore, the activation of toll-like receptor (TLR) 4 by lipopolysaccharide (LPS) from gut Gram-negative bacteria has been shown to promote HCC development in mice treated with DEN plus CCl_4[42]. 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 HCC development[25]. Similar reduction of HCC development was also observed in germ-free mice treated with DMBA at the neonatal stage followed by HFD feeding (Hara's lab, unpublished results), suggesting that the obesity-induced alteration of gut microbiota is likely to provoke the senescence-like features in HSCs. Since we were unable to determine the role of LPS in our experimental setting, and a treatment with vancomycin (VCM), an antibiotic that preferentially targets Gram-positive bacteria, alone was sufficient to block the appearance of senescent HSCs and HCC development[25], 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 a HFD to mice[25]. Together, these results led us to speculate that the obesity-associated increase of gut Gram-positive bacteria may

promote HCC 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 (LC-MS), and found that the level of deoxycholic acid (DCA), a secondary bile acid, was substantially increased by the HFD feeding, and was reduced by the antibiotic treatment[25]. The liver generates bile acids and are released into the intestines to digest and absorb fatty foods. The bile acids are then absorbed by the intestines and passed back to the liver. Some gut bacteria, such as *Clostridium* clusters XI and XIVa (VCM-sensitive Gram-positive bacteria), chemically alter these bile acids to secondary bile acids, which are toxic to animals and even to certain gut bacteria depending on the concentration[43]. The DCA is the most common secondary bile acid and carries the fat in the same way as original (primary) bile acids and follows the same path from gut to liver[43]. But, it can cause DNA damage[44]. Moreover, in addition to colon carcinogenesis, DCA has been shown to enhance liver carcinogenesis[45]. We therefore next focused on DCA. Interestingly, DFAIII-mediated suppression of the 7α-dehydroxylation of primary bile acids, the metabolic pathway for DCA production, or UDCA (ursodeoxycholic acid)-induced stimulation of bile acid secretion reduced HCC development, and markedly decreased the number of senescent HSCs in obese mice treated with DMBA at the neonatal stage[25]. Conversely, prolonged treatment with DCA promoted HCC development in lean mice treated with DMBA at the neonatal stage (Hara's lab, unpublished results), consistent with previous reports that DCA has the potential to cause DNA damage and enhance liver carcinogenesis in rodents[44, 45].

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[25]. 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 hiranonis* and *Clostridium sordellii*[43]. 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.

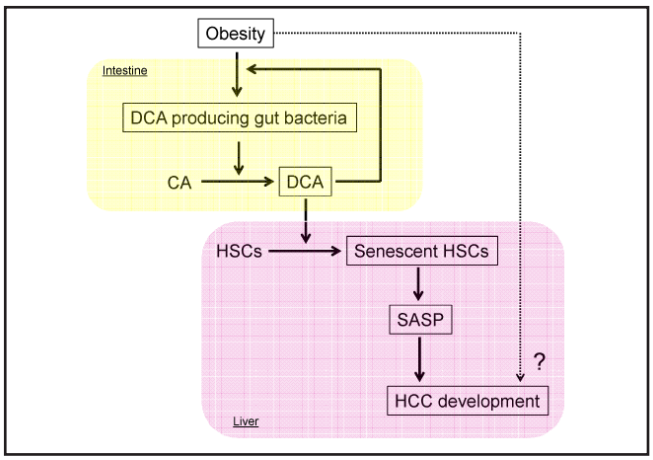

Fig. 2 Model of obesity induced HCC development via SASP
Dietary or genetic obesity increases DCA producing gut bacteria, thereby causing promotion of DCA production in intestinal tract. Elevated levels of DCA further increases DCA producing gut bacteria and provoke cellular senescence and SASP in HSCs, which in turn, secretes various inflammatory and tumor promoting factors in liver. This event, together with the activation of other pathway(s) by obesity, results in the promotion of HCC development.

SASP in human NASH-associated liver cancer

To further support and extend our murine data to human biology, we asked whether our observations could be applied to humans. Note that at least five studies have found that obesity increases (by 1.5- to 4- fold) the risk of liver cancer[3, 46-49]. Moreover, in the setting of chronic hepatitis B or C infection, coexisting obesity has been shown to increase the risk for HCC by more than 100-fold[50]. It should also be noted that obesity is a major cause of non-alcoholic steatohepatitis (NASH) and NASH is a risk factor for liver cancer[51, 52]. 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 is reportedly increased in obese people[40] and high fat consumption causes higher fecal DCA concentrations in healthy volunteers[53, 54], 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 HCC arising in patients with NASH[25]. These results are in good agreement with our murine data[25], and are consistent with recent reports showing that a certain percentage of NASH-associated HCC

arises from the non-cirrhotic liver[55] and HSCs exhibit pro-inflammatory phenotype rather than fibrogenic phenotype during cellular senescence[56]. Although we do not have a definitive answer yet, these findings, in conjunction with published reports[22, 40, 45, 53, 54, 56, 57], led us to have an idea that senescent HSCs may contribute to at least certain aspects of obesity-associated HCC development in human as well (see model in Fig. 2).

Concluding remarks 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 HCC development in obese mice, residual HCCs were still observed with these perturbations[25]. Furthermore, DCA-feeding alone was insufficient to enhance HCC development in lean mice treated with DMBA at the neonatal stage until at least 30 weeks, although prolonged (55 weeks) DCA-feeding enhanced HCC development (Hara's lab, unpublished results). It is therefore tempting to speculate that one or more additional factor(s) associated with obesity may exist to promote obesity-associated HCC development. We are currently answering this question by identifying additional obesity-associated cancer promoting factors through proteomics and metabolomics approaches together with mouse genetics.

It is also unclear why DCA causes cellular senescence 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[25], and DCA has been shown to enhance colon carcinogenesis[58]. It is therefore possible that DCA may also affect cancers in other organs depending on the biological context. Along the same line, it remains unclear why the population of DCA-producing gut bacteria is increased in obese mice. Interestingly, similar to the HFD feeding, cholic acid (CA) feeding was shown to induce alteration of gut microbial composition and increase of DCA levels in rats[59]. Since DCA producing bacteria, but not most other bacteria, are resistant to CA and DCA, it is most likely that obesity-associated increase of CA and DCA provokes outgrowth of DCA producing bacteria in gut. However, since we have obtained similar results using *Lep*$^{ob/ob}$ mice fed a ND, as compared to the results seen in obese mice fed a HFD[25], it is most likely that obesity, but not the HFD feeding, 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 non-obese 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.

Acknowledgement and Source of funding

The authors thank Naoko Ohtani for helpful discussions and suggestions. This work was supported in part by a grant (CREST) from Japan Science Technology Agency (JST).

Conflict of interests

The authors have a research support from LSI Medience Corporation.

References

1) Kinsella KG: Changes in liofespan expectancy 1900-1990. Am J Clin Nutr. 1992; 55: 1196S-1202S.
2) De Magalhaes JP: How ageing processes influence cancer. Nat Rev Cancer. 2013; 13: 357-365.
3) Bhaskaran K, Douglas I, Forbes H, dos-Santos-Silva I, Leon D, Smeeth L: Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5.24 million UK adults. Lancet. 2013; 384: 755-765.
4) Khandekar MJ, Cohen P, Spiegelman BM: Molecular mechanisms of cancer development in obesity. Nat Rev Cancer. 2011; 11: 886-895.
5) Calle EE, Kaaks R: Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer. 2004; 4: 579-591.
6) Sun B, Karin M: Obesity, inflammation, and liver cancer. J Hepatol. 2012; 56: 704-713.

7) d'Adda di Fagagna F: Living on a break: cellular senescence as a DNA-damage response. Nat Rev Cancer. 2008; 8: 512-522.
8) Kuilman T, Michaloglou C, Mooi WJ, Peeper DS: The essence of senescence. Genes Dev. 2010; 24: 2463-2479.
9) Ohtani N, Hara E: Cellular senescence: Roles and mechanisms of cellular senescence in regulation of tissue homeostasis. Cancer Sci. 2013; 104: 525-530.
10) Campisi J: Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. Cell. 2005; 120: 513-522.
11) Collado M, Serrano M: Senescence in tumours: evidence from mice and humans. Nat Rev Cancer. 2010; 10: 51-57.
12) Adams PD: Healing and Hurting: Molecular mechanisms, functions, and pathologies of cellular senescence. Mol Cell. 2009; 36: 2-14.
13) Perez-Mancera PA, Young ARJ, Narita M: Inside and out: the activities of senescence in cancer. Nat Rev Cancer. 2014; 14: 547-558.
14) Coppé JP, Patil CK, Rodier F, Sun Y, Muñoz DP, Goldstein J. et al: Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol. 2008; 6: 2853-2868.
15) Rodier F, Campisi J: Four faces of cellular senescence. J Cell Biol. 2011; 192: 547-556.
16) Kuilman T, Peeper DS: Senescence-messaging secretome: SMS-ing cellular stress. Nature Rev Cancer. 2009; 9: 81-94.
17) Acosta JC, O'Loghlen A, Banito A, et al: Chemokine signaling via the CXCR2 receptor reinforces senescence. Cell. 2008; 133: 1006-1018.
18) Kuilman T, Michaloglou C, Vredeveld LC, et al: Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. Cell. 2008; 133: 1019-1031.
19) Ventura A, Kirsch DG, McLaughlin ME, et al: Restoration of p53 function leads to tumour regression in vivo. Nature. 2007; 445: 661-665.
20) Xue W, Zender L, Miething C, et al: Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. Nature. 2007; 445: 656-660.
21) Kang TW, Yevsa T, Woller N, et al: Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. Nature. 2011; 479: 547-551.
22) Park EJ, Lee JH, Yu GY, He G, Ali SR, Holzer RG, et al: Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. Cell. 2010; 140: 197-208.
23) Serrano M, Lee H, Chin L, Cordon-Cardo C, Beach D, DePinho RA: Role of the INK4a locus in tumor suppression and cell mortality. Cell. 1996; 85: 27-37.
24) Ohtani N, Imamura Y, Yamakoshi K, Hirota F, Nakayama R, Kubo Y, et al: Visualizing the dynamics of p21Waf1/Cip1 cyclin-dependent kinase inhibitor expression in living animals. Proc Natl Acad Sci U S A. 2007; 104: 15034-15039.
25) Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, et al: Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature. 2013; 499: 97-101.
26) Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, et al: A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci U S A. 1995; 92: 9363-9367.
27) Narita M, Nũnez S, Heard E, Narita M, Lin AW, Hearn SA, et al: Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell. 2003; 113: 703-716.
28) Lee BY, Han JA, Im JS, Morrone A, Johung K, Goodwin EC, et al: Senescence-associated β-galactosidase is lysosomal β-galactosidase. Aging Cell. 2006; 5: 187-195.
29) Di Micco R, Sulli G, Dobreva M, Liontos M, Botrugno OA, Gargiulo G, et al: Interplay between oncogene-induced DNA damage response and heterochromatin in senescence and cancer. Nat Cell Biol. 2011; 13: 292-302.
30) Kosar M, Bartkova J, Hubackova S, Hodny Z, Lukas J, Bartek J: Senescence-associated heterochromatin foci are dispensable for cellular senescence, occur in a cell type- and insult-dependent manner and follow expression of p16(ink4a). Cell Cycle. 2011; 10: 457-468.
31) Kennedy AL, McBryan T, Enders GH, Johnson FB, Zhang R, Adams PD: Senescent mouse cells fail to overtly regulate the HIRA histone chaperone and do not form robust Senescence Associated Heterochromatin Foci. Cell Div. 2010; 5: 16.
32) Imai Y, Takahashi A, Hanyu A, Hori S, Naka K, Hirao A, et al: Crosstalk between the Rb pathway and AKT signaling forms aquiescence-senescence switch. Cell

Rep. 2014; 7: 194-207.
33) Orjalo AV, Bhaumik D, Gengler BK, Scott GK, Campisi J. Cell surface-bound IL-1alpha is an upstream regulator of the senescence-associated IL-6/IL-8 cytokine network. Proc Natl Acad Sci U S A. 2009; 106: 17031-17036.
34) Acosta JC, Banito A, Wuestefeld T, Georgilis A, Janich P, Morton JP, et al: A complex secretory program orchestrated by the inflammasome cobntrols paracrine senescence. Nat Cell Biol. 2013; 15: 978-990.
35) Yang L, Jung Y, Omenetti A, Witek RP, Choi S, Vandongen HM, et al: Fate-mapping evidence that hepatic stellate cells are epitherial progenitors in adult mouse livers. Stem Cells. 2008; 26: 2104-2113.
36) Sato Y, Murase K, Kato J, Kobune M, Sato T, Kawano Y, et al: Resolution of liver cirrhosis using vitamin A-coupled liposomes to deliver siRNA against a collagen-specific chaperone. Nat Biotechnol. 2008; 26: 431-442.
37) Cougnoux A, Dalmasso G, Martinez R, Buc E, Delmas J, Gibold L, et al: Bacterial genotoxin colibactin promotes colon tumour growth by inducing a senescence-associated secretory phenotype. Gut. 2014; 63: 1932- 1943.
38) Lujambio A, Akkari L, Simon J, Grace D, Tschharganeh DF, Bolden JE, et al: Non-cell autonomous tumor suppression by p53. Cell. 2013; 153: 449-460.
39) Pribluda A, Elyada E, Wiener Z, Hamza H, Goldstein RE, Biton M, et al: A senescence- inflammatory switch from cancer-inhibitory to cancer-promoting mechanism. Cancer Cell. 2013; 24: 242-256.
40) Ley RE, Turnbaugh PJ, Klein S, Gordon JI: Microbial ecology: human gut microbes associated with obesity. Nature. 2006; 444: 1022-1023.
41) Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI: An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006; 444: 1027-1031.
42) Dapito DH, Mencin A, Gwak GY, Pradere JP, Jang MK, Mederacke I, et al: Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. Cancer Cell. 2012; 21: 504-516.
43) Ridlon JM, Hylemon PB. Identification and characterization of two bile acid coenzyme A transferases from Clostridium scindens, a bile acid 7alpha-dehydroxylating intestinal bacterium. J Lipid Res. 2012; 53: 66-76.
44) Payne CM, Weber C, Crowley-Skillicorn C, Dvorak K, Bernstein H, Bernstein C, et al: Deoxycholate induces mitochondrial oxidative stress and activates NF-kappaB through multiple mechanisms in HCT-116 colon epithelial cells. Carcinogenesis. 2007; 28: 215-222.
45) Kitazawa S, Denda A, Tsutsumi M, Tsujiuchi T, Hasegawa K, Tamura K, et al: Enhanced preneoplastic liver lesion development under selection pressure' conditions after administration of deoxycholic or lithocholic acid in the initiation phase in rats. Carcinogenesis. 1990; 11: 1323-1328.
46) Calle EE, Rodriguez C, Walker-Thunmond K, Thun MJ: Overweight, obesity, and mortality from cancer in a proppectively studies cohort of U.S. adults. N Engl J Med. 2003; 348: 1625-1638.
47) Samanic C, Gridley G, Chow WH, Lubin J, Hoover RN, Fraumeni JF Jr: Obesity and cancer risk among white and black United States veterans. Cancer Causes Control. 2004; 15: 35-43.
48) Møller H, Mellemgaard A, Lindvig K, Olsen JH: Obesity and cancer risk: a Danish record-linkage study. Eur J Cancer. 1994; 30A: 344-350.
49) Wolk A, Gridley G, Svensson M, Nyrén O, McLaughlin JK, Fraumeni JF, et al: A prospective study of obesity and cancer risk (Sweden). Cancer Causes Control. 2001; 12: 13-21.
50) Chen CL, Yang HI, Yang WS, Liu CJ, Chen PJ, You SL, et al: Metabolic factors and risk of hepatocellular carcinoma by chronic hepatitis B/C infection: a follow-up study in Taiwan. Gastroenterology. 2008; 135: 111-121.
51) Haslam DW, James WPT: Obesity. Lancet. 2005; 366: 1197-1209.
52) Torres DM, Harrison SA: Nonalcholic steatohepatitis and noncirrhotic hepatocellular carcinoma: Fertile soil. Semin Liver Dis. 2012; 32: 30-38.
53) Rafter JJ, Child P, Anderson AM, Alder R, Eng V, Bruce WR: Cellular toxicity of fecal water depends on diet. Am J Clin Nutr. 1987; 45: 559-563.
54) David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al: Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014; 505: 559-563.
55) Torres DM, Harrison SA: Nonalcholic steatohepatitis and noncirrhotic hepatocellular carcinoma: Fertile soil. Semin Liver Dis. 2012; 32: 30-38.
56) Schnabl B, Purbeck CA, Choi YH, Hagedorn CH, Brenner D: Replicative senescence of activated human hepatic stellate cells is accompanied by a pronounced inflammatory but less fibrogenic phenotype. Hepatology. 2003; 37: 653-664.

57) Newell P, Toffanin S, Villanueva A, Chiang DY, Minguez B, Cabellos L, et al: Ras pathway activation in hepatocellular carcinoma and anti-tumoral effect of combined sorafenib and rapamycin in vivo. J Hepatol. 2009; 51: 725-733.
58) McGarr SE, Ridlon JM, Hylemon PB: Diet, anaerobic bacterial metabolism, and colon cancer: a review of the literature. J Clin Gastroenterol. 2005; 39: 98-109.
59) Islam KBMS, Fukiya S, Hagio M, et al: Bile acidis a host factor that regulates the composition of the cecal microbiota in rats. Gastroenterol. 2011; 141: 1773-1781.