Biochemical and Biophysical Research Communications 463 (2015) 344–350

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Effect of hyperglycemia on hepatocellular carcinoma development in diabetes

Yasuhiro Niwa [a], Kota Ishikawa [a], Masatoshi Ishigami [b], Takashi Honda [b], Koichi Achiwa [b], Takako Izumoto [a, c], Ryuya Maekawa [a], Kaori Hosokawa [a], Atsushi Iida [a], Yusuke Seino [d], Yoji Hamada [d], Hidemi Goto [b], Yutaka Oiso [a], Hiroshi Arima [a], Shin Tsunekawa [a, *]

[a] Department of Endocrinology and Diabetes, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan
[b] Department of Gastroenterology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan
[c] Department of Oral and Maxillofacial Surgery, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan
[d] Metabolic Medicine, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan

ARTICLE INFO

Article history:
Received 15 May 2015
Accepted 17 May 2015
Available online 28 May 2015

Keywords:
Hyperglycemia
Hepatocellular carcinoma
7,12-Dimethylbenz (a) anthracene
Insulin resistance
High-starch diet
Gut microbiota

ABSTRACT

Compared with other cancers, diabetes mellitus is more closely associated with hepatocellular carcinoma (HCC). However, whether hyperglycemia is associated with hepatic carcinogenesis remains uncertain. In this study, we investigate the effect of hyperglycemia on HCC development. Mice pretreated with 7,12-dimethylbenz (a) anthracene were divided into three feeding groups: normal diet (Control), high-starch diet (Starch), and high-fat diet (HFD) groups. In addition, an STZ group containing mice that were fed a normal diet and injected with streptozotosin to induce hyperglycemia was included. The STZ group demonstrated severe hyperglycemia, whereas the Starch group demonstrated mild hyperglycemia and insulin resistance. The HFD group demonstrated mild hyperglycemia and severe insulin resistance. Multiple HCC were macroscopically and histologically observed only in the HFD group. Hepatic steatosis was observed in the Starch and HFD groups, but levels of inflammatory cytokines, interleukin (IL)-6, tumor necrosis factor-α, and IL-1β, were elevated only in the HFD group. The composition of gut microbiota was similar between the Control and STZ groups. A significantly higher number of Clostridium cluster XI was detected in the feces of the HFD group than that of all other groups; it was not detectable in the Starch group. These data suggested that hyperglycemia had no effect on hepatic carcinogenesis. Different incidences of HCC between the Starch and HFD groups may be attributable to degree of insulin resistance, but diet-induced changes in gut microbiota including Clostridium cluster XI may have influenced hepatic carcinogenesis. In conclusion, in addition to the normalization of blood glucose levels, diabetics may need to control insulin resistance and diet contents to prevent HCC development.

1. Introduction

Epidemiological studies have shown that the incidence of various cancers, including cancers of the bladder, breast, colon, endometrium, liver, and pancreas, are significantly elevated in patients with diabetes mellitus (DM) [1,2]. The relative risk of hepatocellular carcinoma (HCC) is the most closely associated with DM [3,4].

The incidence and mortality of HCC ranks the fifth highest in that of all cancers [5]. Important etiological risk factors of HCC include hepatitis viral inflammation, nonalcoholic steatohepatitis, obesity, and smoking. DM is also considered to be associated with a 2.5-fold increased risk for HCC [6,7]. Chronic inflammation, insulin resistance with hyperinsulinemia, and changes in gut microbiota are reported to be associated with carcinogenesis in the livers of DM patients [8–11]. Systemic and local inflammation occurs in obese DM patients [12]. Inflammatory changes in the liver

Abbreviations: DMBA, 7,12-Dimethylbenz (a) anthracene; SASP, senescence-associated secretory phenotype; HFD, high-fat diet; ND, normal diet.

* Corresponding author. Department of Endocrinology and Diabetes, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan.
E-mail address: tsune87@med.nagoya-u.ac.jp (S. Tsunekawa).

http://dx.doi.org/10.1016/j.bbrc.2015.05.066

enhances production of inflammatory cytokines, which can cause cellular proliferation, angiogenesis, and apoptosis suppression, resulting in HCC development [8,13]. Hyperinsulinemia associated with insulin resistance in DM patients promotes protein synthesis, cellular proliferation, and apoptosis suppression, resulting in acceleration of tumor progression [9]. A recent study also reported that gut microbiota, particularly Clostridium cluster XI, play an important role in HCC development through senescence-associated secretory phenotype (SASP) of hepatic stellate cells in high-fat diet (HFD) fed mice [14].

Hyperglycemia is suggested to increase the risk of developing cancer [15]. Hyperglycemia in patients with type 2 DM increases the breast cancer-associated mortality risk [16]. Oxidative stress, hypoxia-inducible factor-1α (HIF-1α), and epigenetic changes induced by hyperglycemia promote tumor progression in pancreatic and breast cancer cell lines [17]. Hyperglycemia can increase the level of serum inflammatory cytokines [18]. These studies suggest that hyperglycemia can potentially promote HCC development.

However, it remains uncertain whether hyperglycemia alone, without any concomitant factors, is associated with carcinogenesis in the liver of DM patients. In this study, we investigate the effect of hyperglycemia on HCC development.

2. Materials and methods

2.1. Animal model

C57Bl/6J mice (Chubu Kagaku Shizai Co. Ltd., Japan), were housed in a temperature-controlled room under conditions of the 12 h light/dark cycle, with free access to food and water. All procedures were performed according to a protocol approved by the Nagoya University Institutional Animal Care and Use Committee. Carcinogenesis was initiated in male mice using 7,12-dimethylbenz (a) anthracene (DMBA; Sigma). Treatment comprised a single application of 50 μL of 0.5% DMBA solution in acetone to the dorsal surface on postnatal day 4. Next, the mother mice and the pups were fed with a normal diet. At 4 weeks old, pups were weaned and subsequently fed with a normal diet, high-starch diet (Starch) or HFD, until euthanization at 40 weeks after DMBA administration. Control groups were fed on a normal diet (ND; CLEA Japan, Osaka, Japan) containing 4.2% fats and 54.6% carbohydrates. A streptozotosin (STZ; Sigma–Aldrich) group was fed with the ND and intraperitoneally (i.p.) injected with STZ. STZ was dissolved in saline and injected at 50 mg/kg body weight daily for 5 days starting at the ninth week of age. This injection helped induce the pancreatic damage causing DM. Starch groups were fed with Starch (CLEA Japan, Osaka, Japan) containing 12.8% fats and 74.1% carbohydrates. HFD group was fed with HFD (HFD32; Chubu Kagaku Shizai Co. Ltd., Japan) containing 32% fats and 29.4% carbohydrates.

2.2. Random blood glucose levels and body weight

Random blood glucose levels and body weight were measured day at 09:00. Blood glucose levels were measured with Antsense III (Horiba, Kyoto, Japan).

2.3. Intraperitoneal glucose tolerance test (IPGTT)

IPGTT was conducted at 32 weeks. After 16 h of fasting, D-glucose, 2 g/kg body weight, was injected i.p. into the treated mice. Plasma glucose levels were then measured at 0, 15, 30, 60, and 120 min after the injection. Plasma insulin levels were measured using a mouse insulin enzyme-linked immunosorbent assay kit (Morinaga, Tokyo, Japan) at the same time points.

2.4. Insulin tolerance test (ITT)

ITT was conducted at 32 weeks. After 6 h of fasting, regular insulin (Humulin U-100; Lilly, Indianapolis, IN), 0.6 g/kg body weight, was injected i.p. into the treated mice. Plasma glucose levels were then measured at 0, 15, 30, 60, and 120 min after the injections.

2.5. Area under the curve (AUC)

AUC for glucose (mg min/dL) and insulin (ng min/mL) was calculated, as described previously [19].

2.6. Triglyceride measurement

The liver tissue was isolated at 40 weeks after DMBA administration and then homogenized with isopropyl alcohol (Wako, Tokyo, Japan). The triglyceride content in the liver tissue was measured using the Triglyceride E-test kit (Wako, Tokyo, Japan).

2.7. Histological analyses

The liver was fixed in 4% paraformaldehyde, sequentially washed thoroughly in PBS, and embedded in paraffin wax. The specimens were sectioned at a thickness of 4 μm and stained with hematoxylin–eosin staining. Steatosis in the liver was assessed by the steatosis score, as described by Kleiner et al. [20], with separate scores for steatosis (0–3). Liver fibrosis was assessed using Sirius red staining, and the areas with positive Sirius red staining were measured using a BZ-9000 fluorescent microscope system (Keyence, Osaka, Japan) in five microscopic fields at a 200-fold magnification. All liver specimens were assessed by two hepatologists blinded to the identities of the study groups.

2.8. RNA expression of cytokine

Total RNA was extracted from each liver and HCC using the RNeasy® Plus Mini kit from Qiagen (Valencia, CA, USA). RNA expression of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and IL-1β relative to GAPDH was quantified using Power SYBR Green RNA-to-CTTM 1-Step kit in a 7300 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The following primer sets were used: For IL-6, forward primer: 5′-CCGGAGAGGAGACTTC ACAG-3′ and reverse primer: 5′-TCCACGATTTCCCAGAGAAC-3′. For TNF-α, forward primer: 5′-TATGGCTCAGGGTCCAACTC-3′ and reverse primer: 5′-CTCCCTTTGCAGAACTCAGG-3′. For IL-1β, forward primer: 5′-GATCCACACTCTCCAGCTGCA-3′ and reverse primer: 5′-CAACCAACAAGTGATATTCT-3′. For GAPDH, forward primer: 5′-CCAATGTGTCCGTCGTGGAT-3′ and reverse primer: 5′-TGCTGTTGAAGTCGCAGGAG-3′.

2.9. Bacterial 16S rRNA amplification sequencing and analysis

Fecal samples were collected from mice at 40 weeks after DMBA administration. DNA was extracted from the feces and the large intestinal microbiota was analyzed using terminal restriction fragment length polymorphisms (T-RFLP), as described previously [21–23].

2.10. Determination of the copy number of fecal bacteria

The copy number of Clostridium cluster XI was calculated from the standard curve of known bacterial copy number by quantitative real-time PCR of 16S rRNA gene using 5′-TGACGGTACYYNRKGAGG AAGCC-3′ and 5′-ACTACGGTTRAGCCGTAGCCTTT-3′ primers as described previously [24].

2.11. Statistical analysis

Data is expressed as the mean ± standard error. Comparisons among quantitative variable groups were performed using analysis of variance with the Tukey post-hoc test via Graph Pad Prism (v.6.03; Graph Pad Software, San Diego, CA, USA). *P*-value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Incidence of hepatocellular carcinoma

We examined the effect of glucose intolerance on HCC development in the STZ, Starch, or HFD group mice in comparison with the Control group mice. All the mice were pretreated with DMBA on the postnatal day 4.

Multiple HCC were macroscopically observed only in the HFD group (Fig. 1A). The incidence of HCC was 100% in the HFD group (Fig. 1B). The HFD group showed a significant increase in liver weight compared with the other groups (Fig. 1C).

3.2. Glucose intolerance and insulin resistance

The STZ, Starch, and HFD groups showed significantly greater mean random blood glucose levels than the Control group for the 30-week period. The random blood glucose levels in the STZ group were significantly greater than those in the other groups (Fig. 2A). The mean body weight of both the Starch and HFD groups was significantly greater than and that of STZ group was significantly lower than the Control group over the 30-week period (Fig. 2B). IPGTT study at 32 weeks showed that the plasma glucose levels were significantly higher in STZ, Starch, and HFD groups than in the Control group. The glucose AUC during IPGTT in STZ group was the highest among all groups as shown in Fig. 2C [Glucose AUC (mg min/dl): 33,870 ± 2162.8 (Control), 55,612 ± 4381.9 (STZ), 43,029 ± 3317.4 (Starch) and 39,863 ± 4397 (HFD)]. The plasma insulin levels during IPGTT were significantly higher in the Starch and HFD groups and lower in the STZ group than in the Control group. The insulin AUC in the HFD group was significantly higher than that in the other groups as shown in Fig. 2D [Insulin AUC (ng min/ml): 29,313 ± 4780.5 (Control), 9834.8 ± 5396.1 (STZ), 42,050 ± 16,354 (Starch) and 1,25,898 ± 13,547 (HFD)]. ITT at 32 weeks showed insulin resistance in the HFD and Starch groups but not in the STZ or Control group (Fig. 2E). The insulin resistance in the HFD group was more remarkable than that in the Starch group [percentages of blood glucose at 60 min after insulin injection: 30.6% ± 2% (Control), 34.8% ± 1% (STZ), 55.1% ± 3% (Starch), and 71.8% ± 4% (HFD)]. These data suggest that hyperglycemia rarely influences HCC development; nevertheless, insulin resistance may be associated with HCC development.

3.3. Histopathological observations in the liver and HCC

Histopathological analysis showed that the HCC development was observed only in the HFD group with hematoxylin–eosin staining and were not observed in the other groups at 40 weeks after DMBA administration (Fig. 3A). The tissue sections prepared from the HFD group showed the development of HCC with distinct nuclear atypia and pleomorphism (Fig. 3B), which is in a clear contrast to non-HCC area, as described previously [11]. Macroscopic and histopathological observations indicated hepatic steatosis in the Starch and HFD groups (Fig. 3A). The steatosis score was significantly increased in the Starch and HFD groups but not in the STZ or Control group (Fig. 3C). The score in the HFD group was significantly higher than that in the Starch group. The liver triglyceride content was significantly increased in the Starch and HFD groups compared with that in the Control and STZ groups (Fig 3D). The hepatic triglyceride content in the HFD group was

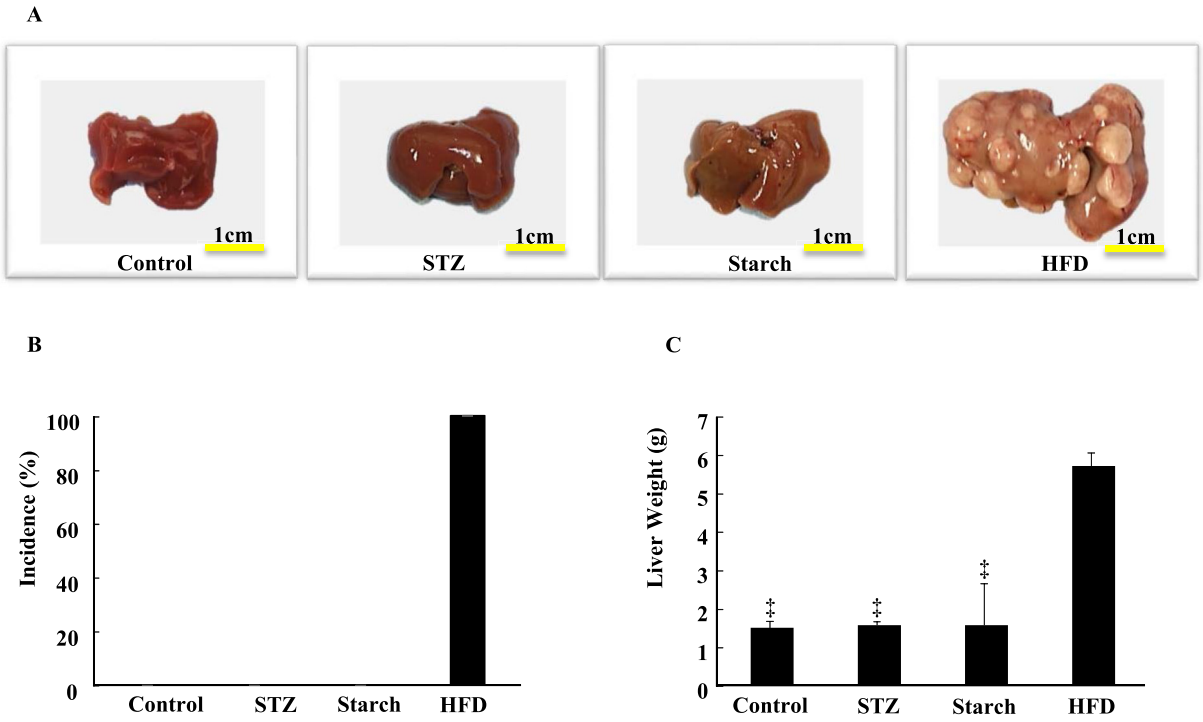

Fig. 1. Incidence of HCC. (A) The livers of mice in the Control, STZ, Starch, and HFD groups at 40 weeks after administration of 50 mL 0.5% solution of DMBA at 4 days of age. (B) Liver weights of each group at 40 weeks after the administration. (C) Tumor incidence in the livers of each group at 40 weeks. The data are expressed as mean ± standard error (n = 6). ‡P < 0.05, vs. HFD.

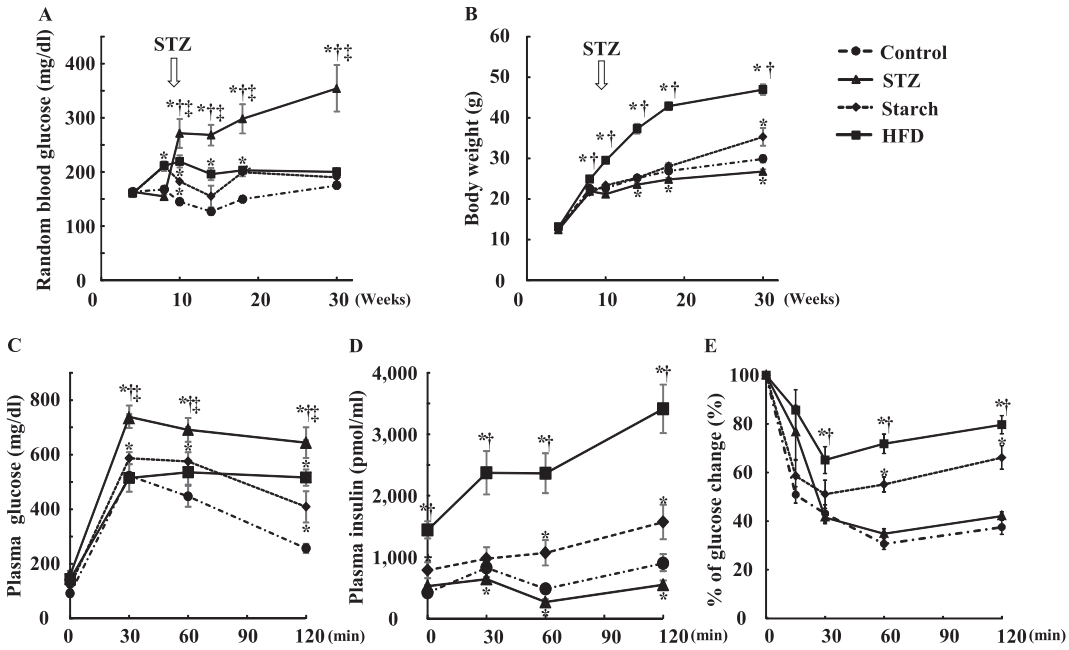

Fig. 2. Glucose tolerance and insulin resistance. (A) Random blood glucose levels and (B) body weights of each mouse of all groups were measured for 30 weeks. (C) Plasma glucose levels and (D) insulin plasma levels of each mouse of all groups during IPGTT were measured at 32 weeks after DMBA administration. (E) Percentages of glucose change in each group were measured by ITT at 32 weeks after DMBA administration. Data are expressed as mean ± standard error (n = 6). $^{*}P < 0.05$, vs. Control. $^{\dagger}P < 0.05$, vs. Starch. $^{\ddagger}P < 0.05$, vs. HFD.

significantly higher than in the Starch group. Sirius red staining demonstrated that the percentage of Sirius red-positive areas in the HFD group was significantly higher than that in all other groups (Fig. 3E). The hepatic fibrosis in the HFD group was denoted as light hepatic fibrosis. These results suggest that the degree of hepatic steatosis may be associated with HCC development.

3.4. *Inflammatory cytokine expression in the liver and change in gut microbiota*

Furthermore, we examined inflammatory state of the liver. mRNA expression levels of inflammatory cytokines including IL-6, TNF-α, and IL-1β in the liver at 40 weeks after DMBA administration were significantly higher in the HFD group than in the other groups. mRNA expression levels of these inflammatory cytokines in tumor tissues were significantly higher than in normal tissues of the HFD group mice (Fig 4A, B, C). The Starch group showed hepatic steatosis but not inflammatory cytokine level elevation or tumorigenesis. These differences between the Starch and HFD groups may be associated with not only insulin resistance but also other factors, such as gut microbiota.

The examination of gut microbiota by the T-RFLP at 40 weeks after DMBA administration showed that the composition of gut microbiota in the STZ group was similar to that of the Control group. The composition of gut microbiota in the HFD group was significantly different from that in the Control group, and the proportion of fecal *Lactobacillus* were remarkably lower in the HFD group than in the other groups (Fig. 4D). The T-RFLP analyses and quantitative real-time PCR of 16S rRNA gene demonstrated that the number of Clostridium cluster XI had markedly increased in HFD group compared with the other groups. Notably, Clostridium cluster XI were completely undetectable in the Starch group (Fig. 4D, E). This data suggest that alterations in gut microbiota, such as an increase in the number of Clostridium cluster XI in HFD, may play important role in HCC development.

4. Discussion

In this study, both the STZ group mice (with severe hyperglycemia) and Starch group mice (with moderate hyperglycemia and hyperinsulinemia) did not show HCC development. Conversely, HCC was observed only in the HFD group mice (with moderate hyperglycemia and severe hyperinsulinemia). These data suggest that hyperglycemia has no effect on HCC development. The differences between the Starch and HFD groups indicate that the degree of insulin resistance and the diet-induced change in gut microbiota may influence HCC development.

Hyperglycemia is reported to promote some carcinogenesis types. HeLa cells transplanted into hyperglycemic nude mice showed an increase in cell proliferation through up-regulation of HIF-1α expression and of vascular endothelial growth factor transcription [25]. Epigenetic modification by hyperglycemia is also reported to accelerate carcinogenesis in breast cancer [26]. However, in this study, hyperglycemia without other concomitant factors did not promote HCC development. Another study reported that hyperglycemia induced by STZ suppressed breast cancer development in DMBA-treated mice [27]. No epidemiological study has shown that the incidence of HCC is elevated in patients with type 1 DM. It remains controversial whether hyperglycemia in patients with type 1 DM significantly promotes other carcinogenesis types [15,28]. These studies suggest that hyperglycemia without other concomitant factors may not affect HCC development.

It is well known that hyperinsulinemia and chronic inflammation in liver is associated with hepatic carcinogenesis [29,30]. Enhanced insulin and insulin-like growth factor 1 signaling promotes protein translation, cell proliferation, and apoptosis suppression through RAS–mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase–AKT pathways, resulting in the promotion of cancer cell growth [30]. Insulin receptor substrate-1-knockout mice fed with HFD were protected against liver tumorigenesis [31]. Therefore, HCC may not develop in the STZ group due

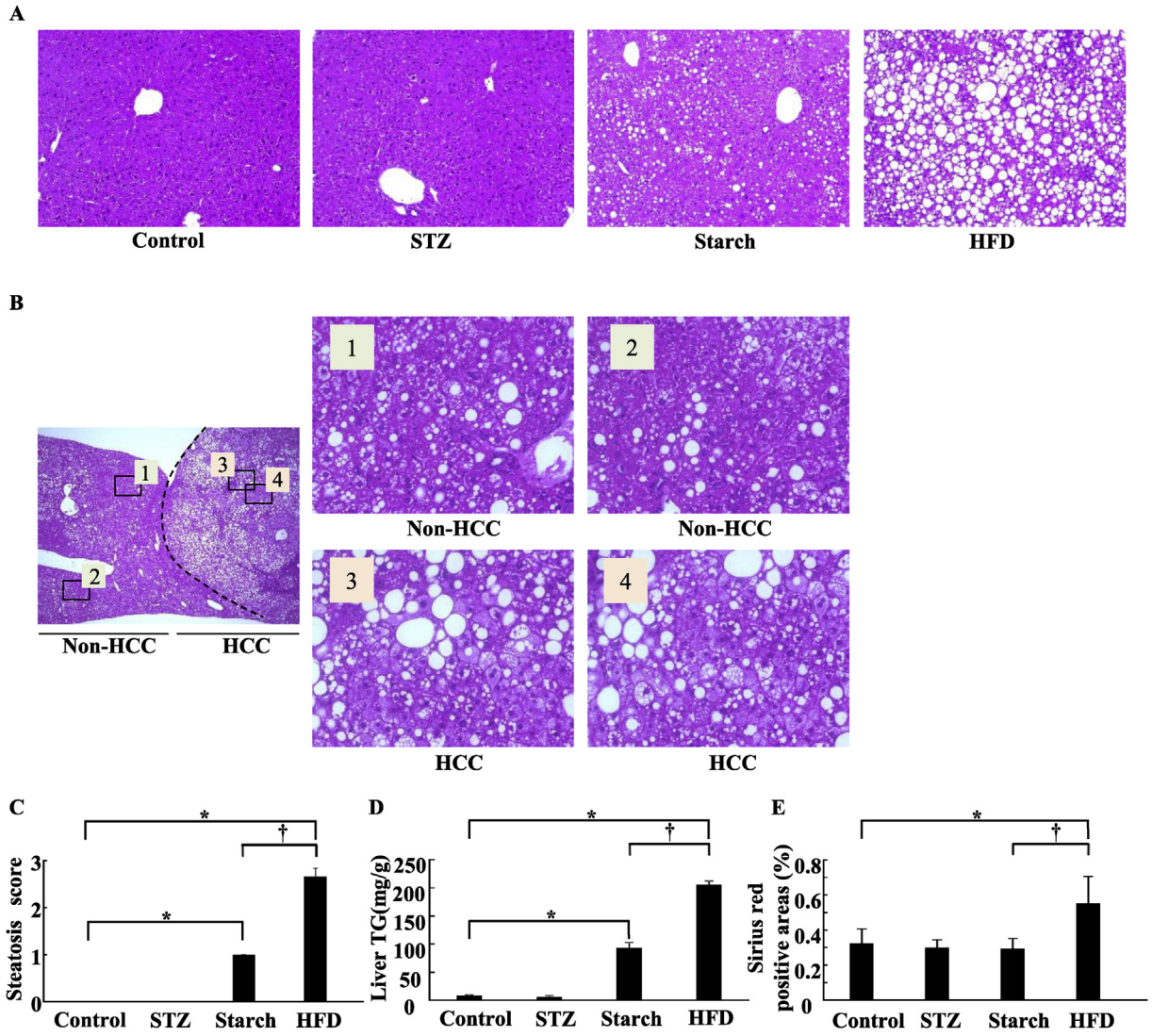

Fig. 3. Histopathological observations in the liver and HCC. (A) Representative histological observations of different experimental mice livers at 40 weeks (hematoxylin–eosin staining; 200× magnification) (B) Representative hematoxylin–eosin-stained images of sections from non-tumor and tumor regions developed in the HFD-fed mice. The box areas in the left panel are magnified (×400) in right panels. (C) Liver steatosis was observed by hematoxylin–eosin staining in each group. The steatosis was designated as the steatosis score. (D) Liver triglycerides in each mouse were determined by a colorimetric assay at 40 weeks after DMBA administration. (E) Liver fibrosis was observed by Sirius red staining in each group. The extent of liver fibrosis was quantified as the percentage of Sirius red-positive areas in each group. The data are expressed as mean ± standard error (n = 3). *P < 0.05, vs. Control. †P < 0.05, vs. Starch.

to hypoinsulinemia; nevertheless, the Starch group with both hyperglycemia and hyperinsulinemia did not have HCC. High levels of inflammatory cytokines including IL-6, TNF-α, and IL-1β enhances various signaling pathways. These pathways includes Janus kinase/Signal transducer activator of transcription 3 pathway and extracellular signal-regulated kinases pathway; it also activates IKKα/nuclear factor-kappa B pathway, resulting in the suppression of apoptosis and cell proliferation in hepatocytes [32–34]. In this study, hepatic inflammation was not observed in the hyperglycemic STZ and Starch groups, although hyperglycemia reportedly induces inflammation in other tissues such as the micro- and macrovasculature through reactive oxygen species or advanced glycation end product level elevation or epigenetic changes [35]. Steatosis is the most common cause of hepatic inflammation [29]. Insulin signaling suppresses lipolysis and promotes lipogenesis in the liver [30]; the STZ group with hypoinsulinemia did not show hepatic steatosis and did not demonstrate liver inflammation or tumorigenesis. However, the Starch group with hepatic steatosis did not demonstrate increase in inflammatory cytokines or tumorigenesis. The difference between the Starch and HFD groups may be attributable to the degree of insulin resistance and level of plasma insulin, but other factors might have influenced hepatic carcinogenesis as well.

A recent study has shown that gut microbiota is involved in HCC development. Clostridium cluster XI, of which the number increased in the HFD group mice, produces the dichloroacetate and plays an important role in HCC development through SASP of hepatic stellate cells [11]. Composition of gut microbiota in the STZ group was highly similar to that of the Control group, suggesting that hyperglycemia without other concomitant factors did not change the proportion of gut microbiota. Clostridium cluster XI in the feces was not detected in the Starch group, which may have contributed to the different incidences of HCC between the HFD and Starch groups, although both groups had hyperglycemia, hyperinsulinemia, and hepatic steatosis. Furthermore, the proportion of fecal *Lactobacillus*, which were reported to decrease in

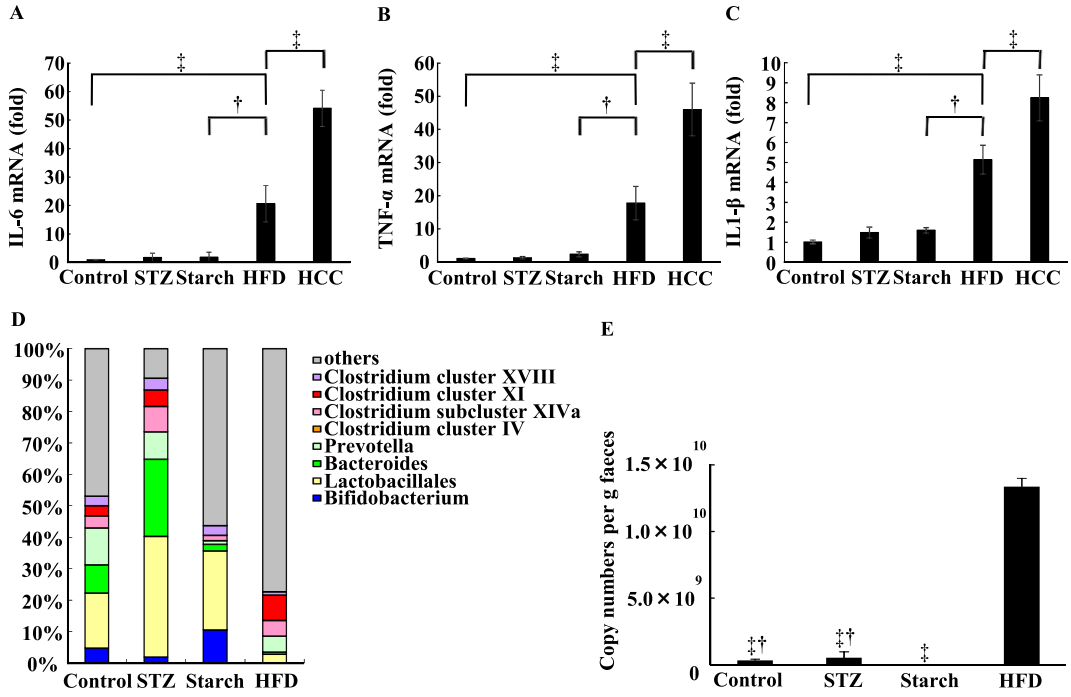

Fig. 4. Inflammatory cytokine expression in the liver and intestinal microbiota (A, B, C) The mRNA expression levels of IL-6, TNF-α, and IL-1β in the tissue of normal livers in each group and the tissue of HCC in the HFD group were determined by quantitative real-time PCR at 40 weeks after the administration of DMBA. (D) The proportion of fecal bacteria of each group were measured by T-RFLP at 40 weeks after DMBA administration. (E) Number of Clostridium cluster XI in feces (1 g) of each group were determined by quantitative real-time PCR at 40 weeks after DMBA administration. Data are expressed as mean ± standard error (n = 3). †P < 0.05, vs. Starch. ‡P < 0.05, vs. HFD.

ulcerative colitis and colorectal carcinoma, were remarkably lower in the HFD group [36]. These combined results suggest that the diet-induced changes in gut microbiota may influence HCC development.

In conclusion, our results suggest that hyperglycemia without any concomitant factors cannot increase the risk of HCC development. The degree of insulin resistance, hepatic steatosis, inflammation in the liver, and particularly, diet-induced changes in gut microbiota may influence HCC development. In addition to the normalization of blood glucose levels, DM patients may need to control insulin resistance and diet contents to prevent HCC development.

Acknowledgments

The authors are grateful to Michiko Yamada (Nagoya University Graduate School of Medicine, Nagoya, Japan) for her technical assistance.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.05.066.

References

[1] K. Shikata, T. Ninomiya, Y. Kiyohara, Diabetes mellitus and cancer risk: review of the epidemiological evidence, Cancer Sci. 104 (2013) 9–14.
[2] W.H. Herman, P. Zimmet, Type 2 diabetes: an epidemic requiring global attention and urgent action, Diabetes Care 35 (2012) 943–944.
[3] M. Inoue, M. Noda, N. Kurahashi, et al., Impact of metabolic factors on subsequent cancer risk: results from a large-scale population-based cohort study in Japan, Eur. J. Cancer Prev. 18 (2009) 240–247.
[4] M. Inoue, N. Kurahashi, M. Iwasaki, et al., Metabolic factors and subsequent risk of hepatocellular carcinoma by hepatitis virus infection status: a large-scale population-based cohort study of Japanese men and women (JPHC Study Cohort II), Cancer Causes Control 20 (2009) 741–750.
[5] H.B. El-Serag, Hepatocellular carcinoma: recent trends in the United States, Gastroenterology 127 (2004) S27–S34.
[6] Y.Y. Wang, S. Huang, J.H. Zhong, et al., Impact of diabetes mellitus on the prognosis of patients with hepatocellular carcinoma after curative hepatectomy, PLoS One 9 (2014) e113858.
[7] B.J. Veldt, W. Chen, E.J. Heathcote, et al., Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus, Hepatology 47 (2008) 1856–1862.
[8] J.P. Bastard, C. Jardel, E. Bruckert, et al., Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss, J. Clin. Endocrinol. Metab. 85 (2000) 3338–3342.
[9] R. Novosyadlyy, D.E. Lann, A. Vijayakumar, et al., Insulin-mediated acceleration of breast cancer development and progression in a nonobese model of type 2 diabetes, Cancer Res. 70 (2010) 741–751.
[10] K. Imajo, K. Fujita, M. Yoneda, et al., Hyperresponsivity to low-dose endotoxin during progression to nonalcoholic steatohepatitis is regulated by leptin-mediated signaling, Cell. Metab. 16 (2012) 44–54.
[11] S. Yoshimoto, T.M. Loo, K. Atarashi, et al., Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome, Nature 499 (2013) 97–101.
[12] J.P. Bastard, M. Maachi, C. Lagathu, et al., Recent advances in the relationship between obesity, inflammation, and insulin resistance, Eur. Cytokine Netw. 17 (2006) 4–12.
[13] E.J. Park, J.H. Lee, G.Y. Yu, et al., Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression, Cell 140 (2010) 197–208.
[14] D. Zipris, The interplay between the gut microbiota and the immune system in the mechanism of type 1 diabetes, Curr. Opin. Endocrinol. Diabetes Obes. 20 (2013) 265–270.
[15] P. Vigneri, F. Frasca, L. Sciacca, et al., Endocr. Relat. Cancer 16 (2009) 1103–1123.
[16] C. Villarreal-Garza, R. Shaw-Dulin, F. Lara-Medina, et al., Herrera, impact of diabetes and hyperglycemia on survival in advanced breast cancer patients, Exp. Diabetes Res. 2012 (2012) 732027.
[17] T.Y. Ryu, J. Park, P.E. Scherer, Hyperglycemia as a risk factor for cancer progression, Diabetes Metab. J. 38 (2014) 330–336.
[18] J. Li, M. Huang, X. Shen, The association of oxidative stress and pro-inflammatory cytokines in diabetic patients with hyperglycemic crisis, J. Diabetes Complicat. 28 (2014) 662–666.
[19] T.M. Wolever, D.J. Jenkins, The use of the glycemic index in predicting the blood glucose response to mixed meals, Am. J. Clin. Nutr. 43 (1986) 167–172.

[20] D.E. Kleiner, E.M. Brunt, M. Van Natta, et al., Design and validation of a histological scoring system for nonalcoholic fatty liver disease, Hepatology 41 (2005) 1313–1321.
[21] K. Nagashima, T. Hisada, M. Sato, et al., Application of new primer-enzyme combinations to terminal restriction fragment length polymorphism profiling of bacterial populations in human feces, Appl. Environ. Microbiol. 69 (2003) 1251–1262.
[22] Y. Nakanishi, K. Murashima, H. Ohara, et al., Increase in terminal restriction fragments of bacteroidetes-derived 16S rRNA genes after administration of short-chain fructooligosaccharides, Appl. Environ. Microbiol. 72 (2006) 6271–6276.
[23] T. Tanigawa, T. Watanabe, K. Otani, et al., Rebamipide inhibits indomethacin-induced small intestinal injury: possible involvement of intestinal microbiota modulation by upregulation of α-defensin 5, Eur. J. Pharmacol. 704 (2013) 64–69.
[24] S. Takahashi, J. Tomita, K. Nishioka, et al., Development of a prokaryotic universal primer for simultaneous analysis of bacteria and archaea using next-generation sequencing, PLoS One 9 (2014) e105592.
[25] Y. Wang, Y.D. Zhu, Q. Gui, et al., Glucagon-induced angiogenesis and tumor growth through the HIF-1-VEGF-dependent pathway in hyperglycemic nude mice, Genet. Mol. Res. 13 (2014) 7173–7183.
[26] J. Park, V.R. Sarode, D. Euhus, et al., Neuregulin 1-HER axis as a key mediator of hyperglycemic memory effects in breast cancer, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 21058–21063.
[27] J.C. Heuson, N. Legros, Influence of insulin deprivation on growth of the 7,12-dimethylbenz(a)anthracene-induced mammary carcinoma in rats subjected to alloxan diabetes and food restriction, Cancer Res. 32 (1972) 226–232.
[28] J.L. Harding, J.E. Shaw, A. Peeters, et al., Cancer risk among people with type 1 and type 2 diabetes: disentangling true associations, detection bias, and reverse causation, Diabetes Care 38 (2014) 264–270.
[29] K. Kato, T. Takamura, Y. Takeshita, et al., Ectopic fat accumulation and distant organ-specific insulin resistance in Japanese people with nonalcoholic fatty liver disease, PLoS One 9 (2014) e92170.
[30] M.J. Khandekar, P. Cohen, B.M. Spiegelman, Molecular mechanisms of cancer development in obesity, Nat. Rev. Cancer 11 (2011) 886–895.
[31] A. Nakamura, K. Tajima, K. Zolzaya, et al., Protection from non-alcoholic steatohepatitis and liver tumourigenesis in high fat-fed insulin receptor substrate-1-knockout mice despite insulin resistance, Diabetologia 55 (2012) 3382–3391.
[32] J. Bromberg, T.C. Wang, Inflammation and cancer: IL-6 and STAT3 complete the link, Cancer Cell. 15 (2009) 79–80.
[33] O. Hammam, O. Mahmoud, M. Zahran, et al., A possible role for TNF-α in coordinating inflammation and angiogenesis in chronic liver disease and hepatocellular carcinoma, Gastrointest. Cancer. Res. 6 (2013) 107–114.
[34] J. Xu, Z. Yin, S. Cao, et al., Systematic review and meta-analysis on the association between IL-1B polymorphisms and cancer risk, PLoS One 8 (2013) e63654.
[35] M.A. Babizhayev, I.A. Strokov, V.V. Nosikov, et al., The Role of oxidative stress in diabetic neuropathy: generation of free radical species in the glycation reaction and gene polymorphisms encoding antioxidant enzymes to genetic susceptibility to diabetic neuropathy in population of type I diabetic patients, Cell. Biochem. Biophys. (2014).
[36] Y.J. Zhang, S. Li, R.Y. Gan, T. Zhou, D.P. Xu, H.B. Li, Impacts of gut bacteria on human health and diseases, Int. J. Mol. Sci. 16 (2015) 7493–7519.