Prevention of Lipid Peroxidation-derived Cyclic DNA Adduct and Mutation in High-Fat Diet-induced Hepatocarcinogenesis by Theaphenon E

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Abstract

Obesity is associated with cancer risk and its link with liver cancer is particularly strong. Obesity causes non-alcoholic fatty liver disease (NAFLD) that could progress to hepatocellular carcinoma (HCC). Chronic inflammation likely plays a key role. We carried out a bioassay in the high-fat diet (HFD)-fed C57BL/6J mice to provide insight into the mechanisms of obesity-related HCC by studying γ-OHPdG, a mutagenic DNA adduct derived from lipid peroxidation. In an 80-week bioassay, mice received a low-fat diet (LFD), high-fat diet (HFD), and HFD with 2% Theaphenon E (TE) (HFD+TE). HFD mice developed a 42% incidence of HCC and LFD mice a 16%. Remarkably, TE, a standardized green tea extract formulation, completely blocked HCC in HFD mice with a 0% incidence. γ-OHPdG measured in the hepatic DNA of mice fed HFD and HFD+TE showed its levels increased during the early stages of NAFLD in HFD mice and the increases were significantly suppressed by TE, correlating with the tumor data. Whole-exome sequencing showed an increased mutation load in the liver tumors of HFD mice with G>A and G>T as the predominant mutations, consistent with the report that γ-OHPdG induces G>A and G>T. Furthermore, the mutation loads were significantly reduced in HFD+TE mice, particularly G>T, the most common mutation in human HCC. These results demonstrate in a relevant model of obesity-induced HCC that γ-OHPdG formation during fatty liver disease may be an initiating event for accumulated mutations that leads to HCC and this process can be effectively inhibited by TE.

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Introduction

Hepatocellular carcinoma (HCC) is responsible for nearly 700,000 deaths each year worldwide (1). The risk factors of HCC include aflatoxin exposure, HBV and HCV infection, alcohol consumption, and obesity (2, 3). Obesity, which causes non-alcoholic fatty liver disease (NAFLD), is strongly linked to HCC and is the fastest growing risk factor for liver cancer in the United States as 40% of its population is obese and 30% suffers from NAFLD (4–6). It is pivotal that we understand how obesity promotes HCC by studying the underlying molecular mechanisms.

Obesity is a chronic inflammatory disease that elicits oxidative stress via reactive oxygen species (ROS) and lipid peroxidation (LPO). Several mechanisms have been proposed for obesity-induced hepatocarcinogenesis, including aberrations in the signaling pathways of IL6, PTEN, NFκB, Hedgehog, TNFα, and STAT3 (7, 8). However, evidence of oxidative stress-induced DNA damage in this process is scarce. An earlier study in human NAFLD showed that 4-hydroxy-2-nonenal modified protein expression, a marker of LPO, as well as 8-oxo-dG, a benchmark of oxidative DNA damage, were elevated (9). We hypothesize that fatty livers caused by high-fat diet (HFD) are
challenged with increased LPO and, consequently, raised the levels of γ-OHPdG, a LPO-derivatized mutagenic DNA adduct, leading to higher mutation loads that set the stage for HCC development and that this process can be prevented by Theaphenon E (TE), a standardized green tea extract formulation.

The accumulation of fat in the liver, known as steatosis, is a risk factor of NAFLD and nonalcoholic steatohepatitis (NASH; ref. 10). Fatty livers increase inflammation and oxidative DNA damage (11) and may progress to fibrosis and cirrhosis due to liver damage caused by LPO and ROS (12). Cirrhosis, a major risk factor for HCC, develops in nearly 2% of the NAFLD population (13). NAFLD can also progress to HCC without cirrhosis (14).

Lipid metabolism changes, genetic alterations, and ROS production are the molecular signatures of fatty liver disease (15, 16). A recent study showed that NAFLD causes selective killing of CD4+ T lymphocytes by ROS in mitochondria due to accumulation of linoleic acid that promotes hepatocarcinogenesis (17). ROS in hepatocytes can be a source of DNA damages. If the damages persist at the critical genes due to lack of repair, they may lead to mutations that drive cancer development. We focused on γ-OHPdG as a mutagenic bulky adduct derived from LPO (18). γ-OHPdG induces primarily G>T and G>A mutations and it preferentially binds at the mutation hotspots of human p53 gene and it is implicated in human cancers (19–21). However, its mutagenicity in vitro may vary and depends on many factors, for example, sequence context (22). In this study, we examined in a HFD-induced HCC model the relationships of γ-OHPdG and the total mutation loads with HCC as a mechanism of obesity-induced hepatocarcinogenesis and demonstrated its prevention by TE.

Materials and Methods

Animal bioassay

A total of 220 male C57Bl/6J mice of three weeks of age were purchased from The Jackson Laboratory (stock no. 000664). The mice were housed and maintained under conventional conditions using micro-Isolator system cage top and polycarbonate mouse cage bottom with the controlled room temperature of 68°–70°F and 68% humidity on a 12-hour light cycle. Mice were randomly divided into three groups; 100 mice in HFD group, 60 mice in low-fat diet (LFD), and 60 in HFD+TE groups. They were fed either a HFD, LFD, or HFD+TE diet starting at four weeks of age. The diet was obtained from Dyets Inc. The HFD (catalog no. 402400) had 60% kcal fat, 20% kcal protein, and 20% kcal carbohydrates. The LFD (#404360) was 12% kcal fat, 29% kcal protein, and 59% kcal carbohydrates. The HFD+TE diet was the same HFD above, supplemented with 2% TE. The 2% TE was chosen based on previous reported bioassays (23). Diet was stored at 4°C and changed weekly. The food consumption was measured weekly and the weekly average consumption was obtained by dividing the number of mice in each cage. Liver tissues from five mice were collected per group at 0, 5, 10, 25, 35, 50, 55, 60, 65, and 70 weeks on the diet and the bioassay was terminated at week 80. Livers were weighed and imaged for gross morphology and sections were formalin-fixed for IHC. Food consumption and body weight were recorded weekly. The bioassay was conducted in conformity with PHS policy and approved by the Association for Assessment and Accreditation of Laboratory Animal Care International–accredited Georgetown University Institutional Review Board and Institutional Animal Care and Use Committee.

Tumors, H&E, Masson Trichrome Staining, and IHC

At each sacrifice during the bioassay, livers were examined for fatty change, fibrosis, and formation of tumors. When tumors were visible they were counted, measured, and those over 2 mm were collected, formalin-fixed, and paraaffin-embedded. Newtissue blocks were made to prepare slides for histologic analysis and confirmation. Tissues were fixed for at least 24 hours in 10% neutral buffered formalin, dehydrated through a graded series of alcohols, cleared in xylene, infiltrated with paraaffin wax, and embedded in paraffin. Tissues were cut in 5-μm sections and placed onto Super Frost Plus charged slides (Thermo Fisher Scientific). For pathology evaluation, routine hematoxylin and eosin (H&E) staining was performed on a Leica Autostainer XL and Masson trichrome staining performed as per standard protocols. For IHC, heat-induced epitope retrieval (HIER) was performed by immersing the tissue sections at 98°C for 20 minutes in Diva Decloaker (BioCare). IHC staining was performed using a horse radish peroxidase (HRP)-labeled polymer (Dako/Agilent K4003) according to the manufacturer’s instructions. Briefly, slides were treated with 3% hydrogen peroxide and 10% normal goat serum for 10 minutes each, and exposed to primary antibodies for Ki67 (Biocare, catalog no. CRM325, 1:50 dilution in VanGough diluent) overnight at 4°C. Slides were exposed to anti-rabbit HRP-labeled polymer for 30 minutes and DAB chromagen (Dako/Agilent) for 5 minutes. Slides were counterstained with hematoxylin (Thermo Fisher Scientific, Harris modified hematoxylin), blued in 1% ammonium hydroxide, dehydrated, and mounted with Acrymount. All washes were performed with TBS with 0.3% Tween. Consecutive sections with the primary antibody omitted were used as negative controls. The hematoxylin and eosin (H&E) slides for liver pathology were evaluated by a board-certified and practicing pathologist. Masson trichrome staining was scored using the scale: 0, normal; 1, expanded; 2, bridging; 3, focal nodule; 4, diffuse (cirrhosis). The diagnosis of HCC was determined on the basis of the presence of standard architectural and/or cytologic atypia including increased thickness of hepatic cords, nuclear enlargement,
prominent nucleoli and multinucleation and the presence of cytoplasmic globules, and abundant Mallory hyaline, particularly in comparison with adjacent nonneoplastic hepatic parenchyma.

Liver function and injury
The levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assessed using Activity Assay Kits (Sigma-Aldrich #MAK052 and MAK055, respectively) according to the manufacturer’s protocol. ALT and AST were detected in the serum of the HFD, LFD, and HFD+TE mice collected and frozen at each time point.

Quantification of γ-OHPdG in mice livers
LC/MS-MS. DNA samples from the flash frozen mouse liver samples were isolated using QIAGEN Blood and Cell Culture Maxi Kit (#13362) following the protocol for liver samples were isolated using QIAGEN Blood and Cell Culture Maxi Kit (#13362) following the protocol for tissue extraction as recommended by the manufacturer. DNA concentration was determined by using a NanoDrop-1000 spectrophotometer (OD ratios at 260/280 nm are 1.8 or higher). γ-OHPdG standards and stable isotope-labeled internal standards were prepared as described in a previous publication (24). Enzymatic hydrolysis of DNA samples was performed using at least 0.2 mg DNA and a portion of each sample was used following hydrolysis for dG analysis as described previously (24). The levels of γ-OHPdG in each DNA sample were determined by LC-MS/MS and expressed as a ratio of γ-OHPdG to dG × 10⁹ (24).

IHC. Livers of 5 mice from each time point (weeks 5, 25, and 50) were processed for staining using a mAb (A2) developed in our laboratory against γ-OHPdG (25). IHC was performed as described for Ki67 with the following modifications: deparaffinization of slides in xylenes was extended by 30 additional minutes, antigen retrieval was performed for 40 minutes in citrate buffer of pH 6.0, and primary antibody was applied for 1 hour at room temperature and applied prior to hydrogen peroxide treatment. Eight to 23 images were randomly selected from each slide. The adduct levels were quantified on the basis of distribution (scale 0–3, respectively, representing 0, 30, 60, and 90% of stained cells) and intensity (from a scale of 0–3 and score 3 is the highest) in each image.

Whole-exome sequencing
Whole-exome sequencing from mouse genomic DNA (gDNA) was performed by Otogeneics using tumor and nontumor liver tissues. Sequencing libraries were prepared using the Agilent SureSelect Target Enrichment System. Paired-end sequencing was performed on Illumina HiSeq2500 platform (PE 100-125, 50×). The liver samples that were analyzed included 4 mice from the HFD group without tumor at the final collection time point and 9 tumors from mice from the HFD group (6 from week 80, 2 from week 70, 1 from week 60). In addition, two blood samples used as background controls from the final time point (one each from HFD and HFD+TE) were sequenced. FASTQ sequencing output was aligned to mouse genome GRCm38/mm10 using bwa-mem, followed by deduplication using Picard and indel realignment using GATK (Broad Institute). Base quality scores were recalibrated (BQSR GATK module) using MGP V5 from the Sanger Mouse Genomes Project to facilitate sensitive variant detection. Variants were called and filtered using LoFreq. P value cutoffs for detection were LoFreq recommended defaults (FDR ≤ 0.05). VCF files were annotated in R using the VariantAnnotation package. Variants detected by LoFreq, at any allele frequency, were included in quantification of the total number of separate mutations, with context sequence provided by GRCm38. Plotting and statistics were produced using custom R code, available on request. VCF files are available from the European Variation Archive (https://www.ebi.ac.uk/eva/, accession code: PRJEB24306). Variant effect prediction was created using the VEP (v86) tool in conjunction with dbSNP (v146) and GENCODE M11 for mouse genome GRCm38.p4 (Supplementary Table S4).

Statistical analysis
To compare outcomes among LFD, HFD, and HFD+TE groups, Student t test, χ² test, and Fisher exact test (for categorical variables) were used. Because the sample size in each group at each timepoint is small, the Wilcoxon rank sum test was used to compare the levels of γ-OHPdG obtained from LC/MS-MS and the regression analysis was employed to determine the levels changes over time in each group. Student t test was used to compare γ-OHPdG values in HFD versus HFD+TE mouse livers obtained from IHC. To determine the significance between LFD, HFD, and HFD+TE samples for liver to body weight ratio, ALT and AST, the Student t test was performed. To calculate the difference in tumor number and size between LFD, HFD, and HFD+TE tumor samples, χ² and Wilcoxon rank sum test are used. A P value of <0.05 was statistically significant.

Results
In an 80-week tumor bioassay, 5 mice from each group were sacrificed at 11 sequential intervals that represent the progression of NAFLD to HCC (Fig. 1A). After normalization, no significant difference in food consumption between HFD, LFD, and HFD+TE mice was observed (Supplementary Fig. S1). HFD+TE group showed the least weight gains, despite consuming a similar amount or more food than the other groups (Fig. 1B). Throughout the bioassay, HFD+TE mice appeared healthy and lean without overt adverse effects. Fig. 1C shows the representative mice at termination. HFD+TE mice generally had a significantly lower liver to body weight ratio than HFD and LFD mice (Fig. 1D).
The livers of LFD and HFD mice showed progressive tissue expansion and discoloration due to lipid accumulation (Fig. 2A). In contrast, livers from HFD+TE mice retained a healthy red color and shiny smooth texture. HFD mice developed steatotic livers at earlier stages. Increasing lipid droplet formation, including macrovesicular and microvesicular steatosis, were seen in LFD and HFD groups, whereas HFD+TE mice showed little or no sign of lipid accumulation (Fig. 2B). In addition, features of NASH, including ballooning of hepatocytes, increased portal and lobular inflammatory infiltrate, neutrophils surrounding lipid-laden hepatocytes, and scattered Mallory hyaline were observed in HFD and LFD mice starting at weeks 25 and 50, respectively (Fig. 2B, inlets). One of 5 mice developed NASH as early as 25 weeks in HFD group and NASH became more frequent at the later weeks (Fig. 2B). The ALT increased after 35 weeks in HFD and LFD mice; however, ALT remained low in HFD+TE mice during the bioassay (Fig. 3A). Similarly, the AST activity was also reduced in HFD+TE mice, specifically at the 35 and 65 weeks (Fig. 3B), although the changes were not as dramatic. Ki67 staining showed increased hepatic cell proliferation during NAFLD in HFD mice compared with HFD+TE mice (Supplementary Fig. S2). These results showed that mice fed HFD+TE had reduced liver toxicity and fatty liver diseases compared with HFD and LFD mice.

HCC was verified by H&E (Fig. 2B). Liver tumors became palpable at week 55 in HFD and LFD groups (Fig. 4A; Supplementary Table S1). At termination, the total tumor numbers between LFD and HFD groups showed no significant difference ($P = 0.9076$), however, the tumor size were significantly lower in LFD than HFD mice ($P = 0.0287$; Fig. 4B and C). The overall tumor incidence was 42% in HFD, 16% in LFD, and 0% in HFD+TE mice.
Prevent Fat Diet-induced DNA Adduct and Liver Cancer by Tea

To verify, we further quantified γ-OH-PdG by IHC method, based on distribution (percentage of positively stained cells) and intensity (score 0–3), using a mAb previously developed in our laboratory (25). The results corroborate the data from LC/MS-MS, showing a small, but significant, increase of adduct in HFD mice from 5 to 25 weeks ($P=0.0478$ distribution), followed by a decline at week 50 ($P=0.0095$ intensity). Although TE treatment showed no difference at week 5, it significantly suppressed γ-OH-PdG levels in HFD group at week 25 ($P=0.03$ and $P=0.0002$, respectively) and week 50 ($P=0.0016$ and $P=0.0006$, respectively) (Fig. 5B). The representative IHC images are shown in Fig. 5C.

To examine the relationships of γ-OH-PdG with somatic mutations, whole-exome sequencing was performed using livers from 13 mice in HFD (9 tumors and 4 normal) and 4 in HFD+TE group (all normal). The overall mutation loads were higher in the tumors from HFD mice than the normal tissues from HFD mice and HFD+TE group ($P=0.0108$ and $P=0.0056$, respectively; Fig. 6A and B). G>A and G>T were over-represented in HCC of HFD mice. When the proportion of specific somatic mutations was compared, G>T was the only mutation significantly decreased in HFD+TE mice ($P=0.0336$; Fig. 6A and C). Examination of the average mutations in each sample based on sequence context shows, again, the mutations in each sample were reduced by TE, and G>T mutations are evidently reduced (Supplementary Fig. S3). However, there is no indication of sequence-dependence in all mutations. In human HCC, base substitution mutations in $\beta$-catenin and p53 genes are common (28, 29). However, no such mutations were detected in the mouse liver tumors. We did identify mutations at codon 61 in HRAS, a mutation hotspot of certain human cancers (30), in the liver tumors obtained from 5 of 9 mice, among them three are G>T mutation.

Discussion

As a disease of chronic inflammation, ROS generation and the related mechanisms are likely to be important in obesity-induced hepatocarcinogenesis. Inflammation-associated signaling pathways have been studied; however, the role of oxidative DNA damages in this process is poorly

Figure 2.
Comparison of liver morphology and histology among the groups. A, The gross morphology of representative nontumor livers from 0, 10, 35, and 70 weeks on the diet. B, H&E staining of livers at 0, 10, 35, 50, and 70 weeks on the diet (20×, scale bar, 200 μm). NASH found in livers of mice from 25 weeks and 50 weeks from HFD and LFD group, respectively, is shown in the inlets. C, Masson trichrome staining of representative sections at 0, 35, 65, and 80 weeks on diet. Blue staining indicates locations of expanded fibrotic tissue. Scale bar, 200 μm.
understood. As fatty livers and inflammation are crucial to elicit oxidative DNA damage, we chose to focus on γ-OHpdG, a well-studied LPO-derived mutagenic adduct. We recently reported that hepatic γ-OHpdG levels not only correlate well with HCC in Xpa\(^{-/-}\) and DEN-exposed mice, it also serves as a biomarker for HCC recurrence in patients after resection (27). We showed that C57BL/6J mice predisposed to obesity and HCC by HFD can be effectively prevented by TE. This model is more relevant than the genetically modified, the methionine- and choline-deficient diet, and chemical-induced HCC models (31, 32). TE treatment significantly lowered adduct levels in mice fed HFD (Fig. 5A and B), correlating well with its inhibition of HCC. These results support the paradigm that higher levels of mutagenic adduct lead to higher mutation loads that increase the risk for HCC. This idea is reinforced by our data that hepatic cell proliferation was increased in NAFLD of HFD mice (Supplementary Fig. S2; ref. 33). Conversely, the diminished steatotic tissues with lowered adduct levels by TE in HFD mice, combining with its effect to decrease hepatic cell proliferation during NAFLD (Supplementary Fig. S2), reduces overall mutation loads and, consequently, lowers HCC incidence.

Two independent methods, LC/MS-MS and IHC, were used to determine the hepatic γ-OHpdG levels. Both confirmed the conclusions that TE suppresses the hepatic adduct levels caused by HFD during the stages of NAFLD. Studies have shown that γ-OHpdG induces predominantly G\(\rightarrow\)T and G\(\rightarrow\)A mutations (19, 20). These mutations also occur frequently in the liver tumors of HFD mice (Fig. 6). The G\(\rightarrow\)T mutations, not commonly associated with other human solid cancers except for lung cancer, are over-represented in human HCC (29). The decreased γ-OHpdG levels by TE is reflected in the lower number of total mutational loads in liver tissues of these mice, particularly G\(\rightarrow\)T mutations. The fact that G\(\rightarrow\)T was the only mutation detected at a significantly lower frequency in liver tissues of mice treated with TE, albeit the sample size is small, implicates its role in HCC. Collectively, these results lend support to the role of γ-OHpdG in hepatocarcinogenesis in this model. It should be mentioned, however, that this is only circumstantial evidence and further investigations are needed to better understand the mechanisms. γ-OHpdG is not the only DNA adduct derived from LPO, more broadly, from oxidative stress. The fact that TE completely blocked HCC in HFD mice, yet only partially blocked hepatic γ-OHpdG formation, may be explained by the assumption that a critical or threshold adduct level exists for HCC initiation or that other mechanisms may be involved, such as the loss of CD4\(^+\) T cells (17), and γ-OHpdG is only partially responsible for HCC development.

Studies have shown that G\(\rightarrow\)T transversion is over-represented somatic mutations in HCC from patients in France that are developed from noncirrhotic livers, implicating the exposure of DNA-damaging agents in hepatocarcinogenesis, which is independent of cirrhotic status (29). A widely recognized liver carcinogen, aflatoxin B1, is an agent that induces G\(\rightarrow\)T mutations (34). However, it is unlikely that this population is exposed to aflatoxin B1. As the causative agents remain elusive, our results may shed some light on the etiologic agent. Whole-genome sequencing of human HCC has identified missense mutations in the β-catenin (33%) and p53 (21%) genes are the most prevalent (28, 29). The more common G\(\rightarrow\)T transversions in human HCC are found in the nontranscribed (NT) strand than in the transcribed (T) strand, indicating that a substantial number of the mutations in HCC are induced by bulky DNA damage (29, 35). However, none of the nine mouse liver tumors examined showed mutations in p53 and β-catenin. Similar observations were made in liver tumors from Xpa\(^{-/-}\) mice (27). This is somewhat unexpected because γ-OHpdG has been shown to preferentially form at mutational hotspots in human p53 (21). A larger sample size may be needed to ascertain the mutation frequencies in these genes or a different set of genes are involved in HCC in these mice. Regardless, our sequencing data revealed variants of other genes that may be informative (Supplementary Table S4). For example, 5 of 9 liver tumors from the HFD group showed mutations in codon...
61 of HRas and 3 of the 5 samples are G>T (Q61K) mutations. The mutation at codon 61 in Hras was reported as a hotspot in certain human cancers, although its relationship with HCC has not been established (30). A recent study identified HRas mutation at codon 61 in HCC in obese mice exposed to DEN (36). Our study is the first to demonstrate HRas mutation at codon 61 of liver tumors in a diet-induced obesity model, suggesting its potential role in hepatocarcinogenesis associated with NAFLD caused by HFD.

Chemopreventive agents, such as metformin and acyclic retinoid, for HFD-induced HCC have been studied (37, 38). Phytochemicals are a rich source of potential cancer chemotherapeutics and chemopreventive agents. EGCG, a major catechin in green tea, reduces body weight gains and lipid accumulation in livers of HFD-fed mice (39). Antioxidant catechins in green tea are known to nullify the adverse effects of LPO (40). The cancer chemoprevention of green tea and its catechins have been studied in chemically induced hepatocarcinogenesis. The total protection
against HCC in mice receiving HFD by TE is remarkable. TE constitutes approximately 70% EGCG, which has been shown to suppress NASH in obese rats (41). TE also reduced body weight gains caused by HFD, possibly related to green tea’s effects on thermogenesis and fat oxidation (42). In this context, a recent study reported that caloric restriction diet inhibits DEN-induced liver tumorigenesis in mice, which may involve multifaceted mechanisms.

Figure 5.
A, γ-OHPdG levels in hepatic DNA measured by LC/MS-MS in mice fed HFD and HFD+TE for 0, 5, 10, 25, 35, and 50 weeks. The Wilcoxon rank sum test shows that the detectible levels of γ-OHPdG in HFD+TE group are significantly lower than that in HFD group (P = 0.03) at week 50. B, Quantification of γ-OHPdG by IHC staining in mouse livers from HFD versus HFD+TE groups based on distribution (% of positively stained cells) and intensity (0–3) at week 5 (P = 0.09 and P = 0.12, respectively), week 25 (P = 0.03 and P = 0.0002, respectively), and week 50 (P = 0.0016 and P = 0.0006, respectively). In HFD mice, an increase of adduct levels from 5 to 25 weeks (P = 0.0478 distribution and P = 0.1699 intensity), followed by a decline at week 50 (P = 0.0190 and P = 0.0095, respectively). In HFD+TE mice, an increase from 5 to 25 weeks (P = 0.0041 distribution), followed by decrease at week 50 (P < 0.0001 and P = 0.0023, respectively). C, Representative IHC staining of livers obtained from mice fed HFD versus HFD+TE at weeks 5, 25, and 50.
The antioxidant effects of catechins may be due in part to increased activities of catalase, superoxide dismutase, and glutathione peroxidase (44). Other possible mechanisms include altered DNA methylation patterns and nuclear factor erythroid 2–related factor 2 (Nrf2) pathway (45). Green tea has also been shown to reduce ROS production and inhibit downstream cell proliferation pathways (46).

The absence of cirrhosis in this model is not unexpected. Several HFD models show no indication of fibrosis and the progressive fibrosis only occurred in HFD-fed mice supplemented with high fructose (26, 47, 48). It appeared that NAFLD is sufficient for HCC development in the HFD model as cirrhosis is not always necessary for HCC in humans and mice (49). Although the model has limitations, the results suggest that targeting NAFLD in obesity by TE may be a viable approach to prevent HCC. A prerequisite for HCC prevention strategy is to identify individuals at high risks. We found elevated hepatic γ-OHPdG levels in patients with early NAFLD followed by a decline during fibrosis and cirrhosis, reminiscent of the present study (50). In a recent study, we also reported that γ-OHPdG predicts recurrence in patients with HCC (27). These results support γ-OHPdG is a useful early biomarker of HCC and its application in identifying individuals with increased HCC risk warrants further investigation.

Because of its increasing prevalence, obesity and NAFLD is believed to be a more significant risk for HCC than diabetes and viral infection combined. An effective and practical preventative strategy based on a better understanding of its mechanism is urgently needed. Dietary-related antioxidants derived from natural sources offer several advantages as cancer-preventive agents because they are relatively low cost, readily available, and, in general, of little toxicity. Our findings that TE, that is well tolerated in patients with hepatitis (51), effectively prevents NAFLD, reduces γ-OHPdG and total mutational loads, and, subsequently, blocks HCC development in HFD-fed mice raise the possibility of its clinical application in preventing HCC associated with obesity.

Figure 6.
A, Number and types of base substitution mutation of liver tumors (T) from nine mice and nontumor liver tissue (N) from four mice on HFD and normal liver tissues from four mice on HFD+TE. B, Dot plot of mutations in the liver tissue of each mouse from HFD (N), HFD(T), and HFD+TE [P = 0.0108 and P = 0.0056 for HFD (T) vs. HFD (N) and HFD (T) vs. HFD+TE normal, respectively, based on Wilcoxon rank sum test]. C, Proportions of mutation types in livers of HFD (T) versus HFD (N) versus HFD+TE. A significant decrease in G>T was found in HFD+TE group as compared with HFD (T; P = 0.0356), but not HFD (T) versus HFD (N) (P = 0.1047) and HFD (N) versus HFD+TE (P = 0.6631), based on Wilcoxon rank sum test. No difference was seen with G>A among the three groups.
Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

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