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Metabonomic Profiling Reveals Cancer Chemopreventive Effects of American Ginseng on Colon Carcinogenesis in *Apc^{Min/+}* Mice

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Abstract

American ginseng (*Panax quinquefolius* L.) is one of the most commonly used herbal medicines in the West. It has been reported to possess significant antitumor effects that inhibit the process of carcinogenesis. However, the mechanisms underlying its anticancer effects remain largely unresolved. In this study, we investigated the cancer chemopreventive effects of American ginseng on the progression of high fat (HF) diet-enhanced colorectal carcinogenesis with a genetically engineered *Apc^{Min/+}* mouse model. The metabolic alterations in sera of experimental mice perturbed by HF diet intervention as well as the American ginseng treatment were measured by gas chromatography time-of-flight mass spectrometry (GC-TOFMS) and liquid chromatography time-of-flight mass spectrometry (LC-TOFMS) analysis. American ginseng treatment significantly extended the life span of the *Apc^{Min/+}* mouse. Significant alterations of metabolites involving amino acids, organic acids, fatty acids and carbohydrates were observed in *Apc^{Min/+}* mouse in sera, which were attenuated by American ginseng treatment and concurrent with the histopathological improvement with significantly reduced tumor initiation, progression and gut inflammation. These metabolic changes suggest that the preventive effect of American ginseng is associated with attenuation of impaired amino acid, carbohydrates and lipid metabolism. It also appears that American ginseng induced significant metabolic alterations independent of the *Apc^{Min/+}* induced metabolic changes. The significantly altered metabolites induced by American ginseng intervention include arachidonic acid, linolelaidic acid, glutamate, docosahexaenoate, tryptophan, and fructose, all of which are associated with inflammation and oxidation. This

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Supporting Information

Figure S1: HPLC analysis of American ginseng extract. (A), Chemical structures of measured ginsenosides; (B), HPLC chromatogram of American ginseng recorded at 202 nm. Ginsenoside peaks: 1, Rg1; 2, Re; 3, Rb1; 4, Rc; 5, Rb2; 6, Rd. This material is available free of charge via the Internet at <http://pubs.acs.org>.

suggests that American ginseng exerts the chemopreventive effects by anti-inflammatory and antioxidant mechanisms.

Graphical Abstract

Keywords

Apc^{Min/+} mice; colorectal cancer; American ginseng; metabolomics; inflammatory cytokines; gas chromatography time-of-flight mass spectrometry; GC-TOFMS; liquid chromatography time-of-flight mass spectrometry; LC-TOFMS

Introduction

Colorectal cancer (CRC) is a very common form of cancer worldwide. Although the incidence and mortality rates of CRC in the United States have declined somewhat in recent years, the numbers remain unacceptably high.¹ It has been estimated that in 2015, there will be 129 978 new CRC cases and 49 700 CRC-related deaths in the United States.² Because CRC patients at advanced stages suffer from a poor prognostic outcome with a low survival rate,³ increased attention has been focused on CRC prevention. It is a particularly relevant public health task to develop more effective interventions, including using natural products, to prevent and treat this disease.

Early intervention against colorectal premalignant lesion with natural products, such as American ginseng, is now one of the most promising approaches to prevent colorectal cancer.^{4–6} American ginseng (*Panax quinquefolius* L.) is one of the most commonly used botanicals in the United States.^{7, 8} Evidence indicated that ginseng not only has cancer prevention potential,^{9, 10} but also has anti-inflammatory effects.^{11, 12} Various data suggested that ginseng reduces inflammation and suppresses colitis by restoring gut homeostasis,¹³ which may play a critical role in cancer prevention and treatment.¹⁴ It has been demonstrated that American ginseng exerts significant antitumor effects in the CRC cell-xenografted nude mouse model.^{15–17} However, to date, mechanistic studies aiming to understand the metabolic alteration in cancer cells by American ginseng leading to cancer prevention have not been reported.

Since limitations of cell culture models, such as mutational complexity and absence of normal microenvironments, controlled *in vivo* studies in animal models have been viewed as

critical tools to test potential preventive and therapeutic strategies.¹⁸ Mutations of the *Adenomatous polyposis coli* (*APC*) gene are responsible for the familial adenomatous polyposis (FAP) syndrome, and are an early causative event in sporadic cancer development.¹⁹ *APC* multiple intestinal neoplasia (*APC^{min}*) was the first heritable mutant *APC* allele to be induced in mice.²⁰ *APC^{min/+}* mice develop numerous intestinal lesions that resemble human FAP and are useful models for investigating malignant transformation in colon tumorigenesis.²¹ Because of its clinical relevance, *Apc^{Min/+}* mice have been used extensively to study the development, treatment and prevention of CRC.^{19, 22, 23} *Apc^{Min/+}* mice fed a Western-style (high fat (HF)) diet developed more adenomas and carcinomas than mice fed with normal chow.²⁴ We hypothesize that American ginseng intervention would be effective at attenuating the HF diet-induced increase in tumor burden in the *Apc^{Min/+}* mouse.

Metabonomics is one of the newly developed approaches to identify biomarkers for diseases, evaluate the effects of drug intervention, as well as metabolic mechanism understanding.²⁵ Metabonomic studies on urine, serum, and tissue of CRC patients have been widely launched and made great progress,^{26–29} which provided the possibility of early diagnosis of CRC cancer. In this study, we used a mass spectrometry based metabonomic approach coupled with histopathology and ELISA analysis to obtain serum metabolite markers associated with high fat (HF) diet-enhanced CRC and to evaluate effects of American ginseng on the progression of HF diet-enhanced CRC carcinogenesis.

Materials and Methods

American Ginseng Extract Preparation and Analysis

The American ginseng extract preparation and analysis were performed according to our published protocol.³⁰ Briefly, the air-dried roots of American ginseng (*P. quinquefolius* L.) purchased from Roland Ginseng, LLC (Wausau, WI, USA) were pulverized, sieved through an 80-mesh screen and extracted with 75% (v/v) ethanol at 95 °C for 4 h each time for three times. The extracting solution was filtered and combined and the filtrate was evaporated under vacuum. The obtained extract was redissolved in water and then extracted three times with water-saturated n-butanol. The n-butanol phase was evaporated at 65 °C under vacuum and lyophilized. Then the ginseng extract was dissolved in methanol and filtered through a membrane syringe filter (0.2-μm) (Millipore Co., Bedford, MA) and subject to HPLC analysis. A Waters Alliance 2960 system (Milford, MA, USA) coupled with a quaternary pump, an automatic injector, and a photodiode array detector (Model 996) were used in this study. The separation was carried out on a Prodigy ODS2 column (250 × 3.2 mm I.D., 5 μm) (Phenomenex, Torrance, CA). The column was eluted with mobile phase A (acetonitrile) and B (water) using a linear gradient of 17.5–21% A over 0–20 min, 21–26% A over 20–23 min, held at 26% A for 19 min, 26–36% A over 42–55 min, 36–50% A over 55–64 min, 50–95% A over 64–66 min, held at 95% A for 3 min, then 95–17.5% A for 3 min, and finally the composition was held at 17.5% A for 8 min. The flow rate was 1 mL/min and the injection volume was 20 μL. All the samples were kept at room temperature during the analysis. The detection wavelength was set at 202 nm. Waters Millennium 32 software was used for peak identification and integration and the content of the constituents was calculated using standard curves of ginsenosides Rb1, Rb2, Rc, Rd, Re, and Rg1.

Animals and Experimental Design

The experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Chicago. Male C57BL/6J-*Apc*^{Min/J} and female C57BL/6J mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) for breeding. Mice were housed under controlled room temperature, humidity and light (12/12 h light/dark cycle) and received chow and tap water *ad libitum*. After weaning, genotyping was carried out by tail biopsy using PCR-based assays to identify *Apc*^{Min/+} mice.³¹ The experimental design was following our initial study.³⁰ All mice consumed standard mouse chow before 6 weeks old. The *Apc*^{Min/+} mice were randomly divided into three groups at 6 weeks of age: (1) model group (HF group), mice that received Western HF diet, (2) LG group, mice that received Western HF diet supplemented with low dose or 100 ppm of the American ginseng extract, equivalent to 10 mg/kg/d, (3) HG group, mice that received Western HF diet supplemented with high dose or 200 ppm of American ginseng extract, equivalent to 20 mg/kg/d. For comparison, a wild type group, which also consumed western HF diet, was used as control (Ctrl group). The Western diet (Harlan Laboratories, Madison, WI) contains 20% fat and includes beef tallow (35 g/kg), lard (30 g/kg) and corn oil (80 g/kg).¹⁷ Five mice in each group were sacrificed at 18 weeks of age. Blood was collected from the posterior vena cava and centrifuged at 10,000×g for 10 min at 4°C. Serum samples were collected and stored at -80°C pending GC-TOFMS and LC-TOFMS analysis. Multiple samples of small intestines and colons were harvested for histological assessment and inflammatory marker analysis. The other mice in each group were used to determine survival rates until 34 weeks of age.

Histological Assessment and ELISA analysis

The small intestine and colon collected from the mice sacrificed at 18 weeks of age were harvested, flushed immediately with ice-cold phosphate-buffered saline (PBS) and slit open longitudinally. Intestinal tumors were examined under a dissection microscope by two independent investigators who were blinded with respect to the treatment group. Colonic and small intestinal samples were fixed in 10% neutral-buffered formalin, embedded in paraffin blocks, and processed by routine hematoxylin and eosin (H&E) staining. The stained sections were subsequently examined for histopathological changes.

ELISA analyses of interleukin-1α (IL-1α), IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-17A, tumor necrosis factor (TNF-α), Interferon gamma (IFN-γ), granulocyte-colony stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF) in colon tissue were performed according to our previous published method.³² Briefly, proteins of the mouse colonic tissue were extracted with RIPA lysis buffer (Thermo Scientific, Hanover Park, IL), adding 10 μL/mL of proteinase inhibitor cocktail and phosphatase inhibitor cocktail (Sigma, St. Louis, MO). ELISA was performed with Multi-Analyte ELISArray Kit (Qiagen, Valencia, CA) containing the above 12 mouse inflammatory cytokines according to the manufacturer's instructions.

Metabolic Profiling of Serum Samples by GC-TOFMS and LC-TOFMS

The metabolite extraction followed our previous published protocols.²⁶ About 25 μL of serum sample was extracted with a 100 μL mixture of methanol and acetonitrile (5:3, v/v,

containing 2-chlorophenylalanine as the internal standard). After being vortexed and centrifuged for 20 minutes at 16,100 g, the supernatant was used for LC-TOFMS and GC-TOFMS analyses.

LC-TOFMS analysis was performed on an Agilent HPLC 1200 system (Agilent Corporation, Santa Clara, CA, USA) coupled with an Agilent model 6220 MSD TOF mass spectrometer (Agilent Corporation, Santa Clara, CA, USA). Metabolites separation was achieved through a 4.6 × 150 mm 5 µm Agilent ZORBAX Eclipse XDB-C18 chromatographic column. The column was maintained at 30°C as a 5 µL aliquot of sample is injected. The mobile phases were water for A and acetonitrile for B in the negative mode (ES-), while 1% of formic acid was added to both solution A and solution B for the positive mode (ES+). The mobile phase program was set as following: 1% B (0–0.5 min), 1% to 20% B (0.5–9.0 min), 20–75% B (9.0–15.0 min), 75–100% B (15.0–18.0 min), 100% B (18–19.5 min), 100% to 1% B (19.5–20.0 min) and isocratic at 1% B (20.0–25.0 min). The parameters for mass spectrometry were as following: capillary voltage, 3500 V for ES+ and 3000V for ES-; nebulizer, 45 psig; drying gas temperature, 325 °C; and drying gas flow 11 L/min. Both plot and centroid data are acquired from the mass range 50 to 1000 Da. Data was centroided, deisotoped, and converted to mzData xml files using the MassHunter Qualitative Analysis Program (vB.03.01) (Agilent Corporation, Santa Clara, CA, USA).

GC-TOFMS data was acquired with an Agilent 7890N gas chromatograph coupled with a Pegasus HT time-of-flight mass spectrometer (Leco Corporation, St Joseph, USA). A 1 µL aliquot of the derivatized solution was injected with splitless mode. Rxi-5 ms capillary column (30 m×250 µm I.D., 0.25-µm film thickness; Restek Corporation, Bellefonte, PA, USA) was used for metabolites separation, with helium as the carrier gas at a constant flow rate of 1.0 mL/min. The temperature settings for injection, transfer interface, and ion source were 260°C, 260°C, and 210°C, respectively. The separation was achieved with the following GC temperature program: 80°C for 2 min, 10°C/min to 220°C, 5°C/min to 240°C, and 25°C/min to 290°C, and kept at 290°C for 8 min. The data was collected with full scan mode (m/z 40–600), and an acquisition rate of 20 spectra/s. Electron impact ionization (70 eV) was used.

Metabonomics Data Analysis

Metabonomic data analysis followed our previous publications.^{26, 33} The data from LC-TOFMS were analyzed with the open source XCMS package (v1.16.3) (<http://metlin.scripps.edu>), to pick, align, and quantify features. The data from GC-TOFMS was processed with ChromaTOF software (v4.22, Leco Co., CA, USA). Compound annotation was performed by comparing the accurate mass and retention time with reference standards available in our laboratory, or comparing the accurate mass with online database such as the Human Metabolome Database (HMDB) for LC-TOFMS data. For GC-TOFMS data, compound annotation was performed by comparing the mass fragments with NIST 08 Standard mass spectral databases with a similarity of more than 70% and finally verified by available reference standards. The annotated compounds from LC-TOFMS and GC-TOFMS were combined and were imported to SIMCA-P software 12.0.1 (Umetrics, Umeå, Sweden) for multivariate statistical analysis. Principle component analysis (PCA) and supervised

partial least squares-discriminant analysis (PLS-DA) were used to compare between groups. Differential metabolites were selected based on the criteria of variable importance in the projection (VIP) >1 in PLS-DA model and q value < 0.05 with a classical one stage false discovery rate (FDR)^{34, 35} on the p value from student's t -test. To systematically understand the metabolism difference between the HF and Ctrl group, we uploaded the metabolite lists and the fold changes of the differentially expressed metabolites onto an IPA server.³⁶ Canonical pathways were generated based on the knowledge sorted in the Ingenuity Pathway Knowledge Base. Fisher's exact test was used to calculate a p -value determining the probability that the association between the metabolites and the canonical pathway was explained.

Results

Saponin Composition in the American Ginseng Samples

The concentrations of the 6 main ginsenosides in American ginseng extract were measured using HPLC (Supplementary Figure 1). These ginsenosides fall into two major groups: the protopanaxadiol group and the protopanaxatriol group. The major constituents of the protopanaxatriol group are Re, followed by Rg1. More than 70% of the ginsenosides are in the protopanaxadiol group, including Rb1, Rb2, Rc, and Rd. The contents of ginsenosides in American ginseng extract are 23.4% (Rb1), 0.3% (Rb2), 1.3% (Rc), 1.6% (Rd), 6.8% (Re), and 0.3% (Rg1).

Effects of American Ginseng on *Apc*^{Min/+} Mouse Survival

As shown in Figure 1, all the mice in the HF model group died at 20 weeks of age. They had blood in the stool, body weight reduction, and evident gut tumorigenesis compared to the ginseng treated groups. At 30 weeks of age, the survival rate of the low dose (LG group) and high dose ginseng (HG group) were 66.7% and 33.3%, respectively. At 34 weeks of age, the survival rates for LG and HG groups were 33.3% and 16.7%, respectively. American ginseng treatment significantly increased survival rate of *Apc*^{Min/+} mice.

Effects of American ginseng on tumor multiplicity

An examination of tumor load showed that the *APC*^{min/+} mice in the HF group had significantly higher tumor load than those in the HF diet plus ginseng groups (LG and HG) in both small intestine and colon (all $p < 0.01$) (Figure 2). American ginseng significantly decreased *APC*^{min/+} mice tumor load when on HF diet.

Effects of American ginseng on gut inflammation

Figure 3A shows representative H&E staining histological sections of experimental animals with different treatments. The histology from HF showed prominent adenomatous change along with inflammatory lesions, such as neutrophil infiltration. The dysplastic changes are greatly reduced in colon slides in the ginseng treated groups, especially the LG group, which was supported by the expression of inflammatory cytokines in gut tissue (Figure 3B). The levels of inflammatory cytokines, such as IL-1 α , IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12, IL-17A, TNF- α , IFN- γ , G-CSF, and GM-CSF are more significantly increased in the gut of *APC*^{min/+} mice in the HF group than the mice in Ctrl group. Oral ginseng administration,

especially the low dose ginseng intervention, significantly reduced the level of these inflammatory cytokines in the intestine tissues.

Metabonomic Variations in *APC^{Min/+}* Mice and Preventive Effect of American Ginseng on HF-Enhanced Carcinogenesis

Since low dose American ginseng has more preventive effect on tumorigenesis, we focused on mice from Ctrl, HF and LG groups for metabonomic analysis. The metabonomic profiles among groups were firstly evaluated with unsupervised PCA, which showed a clear separation between samples from the HF and Ctrl group (2 components, $R^2X=0.736$, Figure 4A), as well as between the LG and Ctrl group (2 components, $R^2X=0.59$, Figure 4C). We then constructed the two-component PLS-DA models between the HF and Ctrl group ($R^2X=0.724$, $R^2Y_{cum}=0.995$, $Q^2_{cum}=0.98$, Figure 4B) and between LG and Ctrl group ($R^2X=0.542$, $R^2Y_{cum}=0.991$, $Q^2_{cum}=0.945$, Figure 4D) to identify the key metabolites associate with *APC* knock out and ginseng treatment. Figures 4B and 4D showed that the scores plots of the PLS-DA model of mice from the Ctrl and LG groups were clearly separated from those of the HF group. Based on the criteria of $VIP > 1$ and p value < 0.05 , 86 metabolites including amino acids, organic acids, fatty acids and carbohydrates were differentially expressed between the HF and Ctrl groups, which showed significant alterations in the HF group, but were attenuated after LG intervention (Figure 5, Table 1). IPA also showed that 24 canonical pathways were significantly altered (Table 2) and 14 amino acids, alanine, tyrosine, proline, threonine, serine, leucine, isoleucine, valine, lysine, methionine, histidine, glycine, glutamine, and involved in the tRNA charging were altered most significantly ($p = 1.55E-09$).

We further identified the differentially expressed serum metabolites in the LG group relative to the HF group by two component PLS-DA model ($R^2X=0.631$, $R^2Y_{cum}=0.998$, $Q^2_{cum}=0.98$, Figure 4E and F). A list of sixty differential metabolites (Table 1) mainly includes fatty acids, amino acids, organic acids, and carbohydrates were identified and 13 of them are different from the metabolites that differed between the HF group and controls.

Discussion

In our previous study, we have shown the inhibitory effect of American ginseng extracts on a colitis-associated CRC model.¹⁷ Here, we investigated the therapeutic and metabonomic effects of ginseng intervention in HF diet treated *Apc^{Min/+}* mice. Our results showed a significantly reduced tumor load in the small intestine and colon in ginseng treated mice compared with those in model group. Our results also showed a significant reduction of inflammatory cytokines including IL-1 α , IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12, IL-17A, TNF- α , IFN- γ , G-CSF, and GM-CSF in intestinal tissues of ginseng treated mice, which is similar to our previous data,³⁰ and supported by the anti-inflammatory effects of ginseng.^{30, 37, 38} These therapeutic effects of ginseng extracts finally resulted in a significant increase of the overall survival time for HF diet treated *Apc^{Min/+}* mice (Figure 1).

In addition to these cytokines, we also analyzed metabolic variations with the serum samples. Our observations showed distinct separation between HF diet treated *Apc^{Min/+}* mice and the wild type ones in the serum, while the intensities of many of those differential

metabolites were normalized to those of wild type, including most of the amino acids, fatty acids, organic acids and carbohydrates after ginseng intervention. The heatmap (Figure 5) generated with the differential metabolites contributing to the separation of the HF group and LG group from the control group indicate less significant fluctuation of metabolite levels in the LG group, suggesting that ginseng could attenuate the metabolic perturbation in *APC^{Min/+}* mice. The inhibition of metabolic alteration by ginseng is consistent with their preventive effects of carcinogenesis as demonstrated by the significantly decreased tumor load and longer survival time in the LG group. However, not all the differentially expressed metabolites in the HF group were attenuated or normalized by ginseng intervention. Therefore, a list of differential metabolites (Table 1) responsible for the separation between the HF group and LG group (Figure 4E and F) was identified. These “LG-induced” markers are different from those differential metabolites in HF group relative to the controls. Some of these significantly altered metabolites in serum, including arachidonic acid, linolelaidic acid, glutamate, docosaheptaenoic acid, tryptophan, and fructose, most of which are associated with inflammation and oxidation. It has been reported that ginseng has potent antioxidant³⁹ and anti-inflammatory^{11, 12} properties and might play an important role in protecting the colon. The significant alteration in inflammation and oxidation-related metabolites suggests that ginseng exerts its chemopreventive effects to decrease tumor load and increase survival time through anti-inflammation and antioxidant mechanisms.

It is reported that amino acid profiles also seem to be important for understanding *APC* mutation and the subsequent Wnt pathway activation.²² Our IPA analysis revealed that the amino acids involved in tRNA charging were the most significantly altered metabolites (Table 2), most of which were up-regulated in the serum of *Apc^{Min/+}* mice compared with wild type ones. However, glutamine was tremendously decreased in the model mice with a FC value of 0.03. Hirayama et al.⁴⁰ reported that most amino acids, except glutamine, were more significantly increased in CRC tissues than in normal colon tissues, indicating that a relationship exists between CRC and amino acids. Interestingly, similar to amino acids, most of the detected sugars and polyols were increased in the *Apc^{Min/+}* mice but the level of glucose significantly decreased in the model mice with a FC value of 0.01, which is supported by other reports that the blood glucose level was significantly decreased in the high fat diet fed *Apc^{Min/+}* mice.²² Glucose and glutamine were main energy sources for cancer cells,⁴¹ and they are associated with inflammation status.⁴² The decreased glucose and glutamine may indicate high energy consumption in the *Apc^{Min/+}* mice. With ginseng intervention, glutamine and glucose were significantly increased in the serum with the FC values of 14.63 and 83.13, respectively, compared to the mice in the HF group. The increased levels of glutamine and glucose may be associated with reduced inflammation. Studies also have shown American ginseng helps increase energy and normalize blood sugar levels.⁴³ One study by Debra Barton et al. revealed that ginseng is a natural energy booster that can help cancer patients overcome fatigue.⁴⁴ An improved endurance and swimming duration time were found with ginseng treatment, specifically ginsenosides Rb1 and Rg1, both of which are present in both Asian and American ginseng.^{45–47} These reports are consistent with our findings that significantly increased levels of glutamine and glucose, as energy source, in HF group were normalized after ginseng treatment. High energy consumption may induce higher amino acid concentration in the serum samples of *Apc^{Min/+}*

mice especially for the branched chain amino acids. In our study, valine, leucine and isoleucine were significantly increased in the HF mice and restored to lower levels with ginseng treatment (Table 1).

The aim of this study was to identify serum metabolite markers associated with HF diet-enhanced CRC and to evaluate effects of American ginseng on the progression of HF diet-enhanced CRC carcinogenesis. However, there are limitations in the current metabonomic study. First, we performed ELISA analysis of the cytokines on tissue samples and metabolomics analysis on the serum samples. We did not perform cytokine assay on the serum samples and metabolomics analysis on the tissue samples, which will provide a more consistent and holistic view of the ginseng effects. Second, we found low dose American ginseng has better preventive effect than high dose American ginseng on tumorigenesis based on the data on survival, tumor multiplicity, and gut inflammation. However, we did not perform metabonomic analysis on the HG group to see the dose effect.

In conclusion, serum metabonomic analysis results indicate that HF diet significantly enhances some of the metabolic perturbations that are associated with *Apc^{Min/+}* mutation and intestine tumor development. Comprehensive metabolic shifts in model mice, including altered serum metabolic profiles involving branched-chain amino acids, fatty acid metabolism, glucose metabolism and TCA cycle, were identified. Low oral dose of American ginseng is able to attenuate the metabolic perturbation in *Apc^{Min/+}* mice, which was supported by gut tissue histology, tumor multiplicity and gut inflammation cytokine data that American ginseng significantly reduced gut inflammation and tumor initiation and progression. It also appears that American ginseng induced significant metabolic alterations independent of the *Apc^{Min/+}* induced metabolic changes. These American ginseng induced alterations include a panel of metabolites that are involved in inflammation and oxidation processes, suggesting that American ginseng exerts the chemopreventive effects through anti-inflammatory and antioxidant mechanisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
American ginseng on *APC^{min/+}* mouse survival.

Figure 2.
Effect of American ginseng on *APC^{min/+}* mouse tumor multiplicity.

Figure 3. Effect of American ginseng on inflammation in *APC^{min/+}* mouse gut tissue. (A) H&E staining of colon tissue. (B) Expression of inflammatory cytokines in colon tissue.

Figure 4. PCA and PLS-DA scores plots for wild-type mice (Ctrl), *APC^{min/+}* mice (HF) and ginseng treated mice (LG) with serum samples. A, PCA scores plots between HF group and Ctrl group; B, PLS-DA scores plots between HF group and Ctrl group; C, PCA scores plots between LG group and Ctrl group; D, PLS-DA scores plots LG group and Ctrl group; E, PCA scores plots between LG and HF group; F, PLS-DA scores plots between LG and HF group.

Figure 5. Heat-map of differential metabolites in the serum samples in Ctrl group, HF group and LG group. Cells of heatmap represent z-score values of metabolites in the corresponding subjects.

Differentiating metabolites between serum samples collected from model mice and ginseng treated mice.

Table 1

Metabolite ^a	Retention time (min)	HF vs. Ctrl			LG vs. Ctrl			HF vs. LG					
		VIP ^b	FC ^c	p ^d	Adjusted p ^e	VIP ^b	FC ^f	p ^d	Adjusted p ^e	VIP ^b	FC ^g	p ^d	Adjusted p ^e
Phosphoethanolamine	15.03	1.05	97.69	3.59E-03	6.68E-03	1.22	41.71	9.74E-04	3.72E-03	1.58	0.1	2.29E-07	1.63E-05
4-Hydroxybutanoic acid*	8.05	1.26	28.4	8.86E-08	2.46E-06	1.12	2.87	5.70E-03	1.45E-02	1.35	0.22	2.21E-03	1.05E-02
Arachidic acid*	21.66	1.2	24.72	6.31E-05	2.45E-04	1.37	24.93	1.37E-06	3.01E-05	1.35	0.22	2.21E-03	1.05E-02
N-formyl-glycine	10.65	1.14	23.15	5.60E-04	1.35E-03	1.08	5.18	8.60E-03	1.94E-02	1.35	0.22	2.21E-03	1.05E-02
Octadecanoic acid*	19.88	1.21	17.79	3.88E-05	1.75E-04	1.37	17.32	1.40E-06	3.01E-05	1.35	0.22	2.21E-03	1.05E-02
9-Tetradecenoic acid	15.24	1.26	15.7	2.65E-07	4.72E-06	1.37	5.91	7.22E-07	1.93E-05	1.55	0.38	8.53E-06	3.65E-04
Aminomalonic acid*	11.33	1.18	14.35	1.52E-04	4.66E-04	1.36	7.37	4.16E-06	7.41E-05	1.23	0.51	9.77E-03	3.22E-02
Uracil*	9.55	1.01	13.65	6.40E-03	1.13E-02	1.19	3.15	1.75E-03	5.56E-03	1.17	0.23	1.66E-02	4.76E-02
Glycine*	9.13	1.23	12.36	9.85E-06	6.80E-05	1.25	5.46	4.64E-04	2.28E-03	1.4	0.44	1.06E-03	6.48E-03
Sucrose*	23.32	1.26	11.99	4.45E-07	7.33E-06	1.1	4.41	6.82E-03	1.68E-02	1.48	0.37	1.39E-04	2.05E-03
Stearoylcarnitine	22.79	1.27	10.77	6.35E-08	2.27E-06	1.3	5.94	4.08E-04	2.13E-03	1.49	0.55	9.90E-05	1.82E-03
Linoleic acid*	19.53	1.22	10.56	2.06E-05	1.16E-04	1.34	9.77	1.24E-05	1.76E-04	1.5	0.41	7.62E-05	1.69E-03
Proline*	8.99	1.25	9.15	5.17E-07	7.38E-06	1.21	3.71	1.23E-03	4.29E-03	1.53	0.33	1.96E-05	6.29E-04
2-Hydroxybutyric acid*	6.51	1.24	8.82	3.16E-06	2.88E-05	1.19	2.92	1.76E-03	5.56E-03	1.49	0.06	1.02E-04	1.82E-03
11-Eicosenoic acid	21.49	1.21	8.35	3.70E-05	1.72E-04	1.27	8.87	3.18E-04	1.79E-03	1.48	0.39	1.53E-04	2.05E-03
5-Hydroxyindoleacetic acid	15.99	1.17	7.57	6.87E-04	1.53E-03	1.15	0.48	4.92E-03	1.30E-02	1.32	0.31	3.50E-03	1.44E-02
Tyrosine*	16.63	1.23	6.85	6.14E-06	5.05E-05	1.26	2.7	3.53E-04	1.94E-03	1.49	0.06	1.02E-04	1.82E-03
Cystine*	20.71	1.19	6.74	1.05E-04	3.46E-04	0.59	2.07	2.22E-01	2.88E-01	1.32	0.31	3.50E-03	1.44E-02
Butyrylcarnitine	12.79	1.27	6.52	2.48E-08	1.06E-06	1.17	6.11	7.34E-03	1.75E-02	1.31	0.52	3.85E-03	1.50E-02
Guanine	4.12	1.2	6.42	3.01E-04	8.23E-04	1.21	3.36	4.12E-03	1.10E-02	1.31	0.52	3.85E-03	1.50E-02
Oleic acid*	19.58	1.21	6.38	3.59E-05	1.72E-04	1.27	6.97	2.74E-04	1.59E-03	1.36	0.48	1.98E-03	9.85E-03
Methionine*	11.91	1.21	6.3	3.65E-05	1.72E-04	1.24	3.02	5.98E-04	2.67E-03	1.32	0.4	3.73E-03	1.48E-02
Malic acid*	11.55	1.19	6.07	9.07E-05	3.08E-04	0.85	2.44	6.31E-02	1.02E-01	1.32	0.4	3.73E-03	1.48E-02
Methylserotonin	21.95	1.04	5.85	4.20E-03	7.68E-03	1.07	4.01	1.04E-02	2.30E-02	1.36	0.48	1.98E-03	9.85E-03
Myristic acid*	15.52	1.22	5.34	1.52E-05	9.54E-05	1.32	4.61	5.55E-05	4.75E-04	1.32	0.4	3.73E-03	1.48E-02
Ethanolamine	8.56	1.25	5.34	1.29E-06	1.62E-05	0.87	3.18	5.40E-02	8.89E-02	1.32	0.4	3.73E-03	1.48E-02
Succinic acid*	9.16	1.22	5.33	1.56E-05	9.56E-05	0.77	11.02	1.02E-01	1.50E-01	1.32	0.4	3.73E-03	1.48E-02

Metabolite ^a	Retention time (min)	HF vs. Ctrl			LG vs. Ctrl			HF vs. LG					
		VIP ^b	FC ^c	p ^d	Adjusted p ^e	VIP ^b	FC ^c	p ^d	Adjusted p ^e	VIP ^b	FC ^c	p ^d	Adjusted p ^e
Aspartic acid*	11.94	1.13	5.15	6.87E-04	1.53E-03	0.4	0.77	4.28E-01	5.03E-01	1.45	0.15	3.90E-04	3.63E-03
Adonitol	14.48	1.11	5.08	1.23E-03	2.58E-03	0.23	0.85	6.53E-01	7.13E-01	1.43	0.17	6.14E-04	4.92E-03
Dodecanoylcarnitine	19.85	1.18	4.98	6.01E-04	1.40E-03	1.26	2.49	1.24E-03	4.29E-03	1.35	0.5	2.20E-03	1.05E-02
Arabinose*	4.13	1.2	4.95	3.08E-04	8.23E-04	1.16	2.58	8.58E-03	1.94E-02	1.29	0.52	5.48E-03	2.02E-02
Alanine*	6.15	1.23	4.86	7.63E-06	6.05E-05	1.24	2.91	6.52E-04	2.79E-03	1.32	0.6	3.49E-03	1.44E-02
Tetradecanoylcarnitine	20.86	1.2	4.86	3.34E-04	8.83E-04	1.13	2.5	1.28E-02	2.66E-02	1.3	0.51	4.53E-03	1.73E-02
Beta-alanine*	10.74	1.2	4.84	6.32E-05	2.45E-04	1.22	2.08	1.02E-03	3.76E-03	1.44	0.43	4.52E-04	4.03E-03
Palmitoleic acid*	17.31	1.2	4.64	6.89E-05	2.59E-04	1.34	4.22	1.74E-05	2.18E-04				
2-aminobutyric acid*	7.18	1.21	4.42	3.93E-05	1.75E-04	1.29	3.84	1.65E-04	1.08E-03				
Phenylalanine*	13.22	1.25	4.29	1.46E-06	1.73E-05	1.21	1.53	1.23E-03	4.29E-03	1.56	0.36	3.05E-06	1.63E-04
Lysine*	16.45	1.2	4.28	5.95E-05	2.40E-04	1.1	4.01	6.90E-03	1.68E-02				
Malonylcarnitine	17.96	1.17	4.11	5.90E-04	1.39E-03	1.14	3.47	7.69E-03	1.79E-02				
Palmitoylcarnitine*	21.85	1.26	4	1.62E-06	1.73E-05	1.2	2.22	5.14E-03	1.34E-02	1.47	0.56	2.16E-04	2.71E-03
2-Hydroxy-3-methylbutyric acid*	7.08	1.23	3.8	9.61E-06	6.80E-05	1.14	2.05	3.92E-03	1.06E-02	1.43	0.54	5.81E-04	4.92E-03
Hexadecanoic acid*	17.59	1.2	3.66	6.41E-05	2.45E-04	1.34	3.63	1.17E-05	1.76E-04				
Pyruvic acid*	5.36	1.22	3.65	1.77E-05	1.05E-04	1	1.78	1.97E-02	3.84E-02	1.4	0.49	9.54E-04	6.19E-03
Citric acid*	15.43	1.22	3.49	2.51E-05	1.25E-04	1.1	4.51	7.07E-03	1.70E-02				
Homogentisic acid	3.28	1.16	3.41	1.15E-03	2.43E-03	1.15	3.33	1.06E-02	2.32E-02				
Acetylcarnitine*	3.91	1.2	3.39	3.07E-04	8.23E-04	1.29	2.68	6.55E-04	2.79E-03				
Oleoylcarnitine	22.01	1.17	3.37	8.40E-04	1.83E-03	1.23	3.78	2.64E-03	7.64E-03				
Lactic acid*	5.56	1.15	2.98	4.59E-04	1.14E-03	0.87	1.37	5.51E-02	9.01E-02	1.35	0.46	2.37E-03	1.08E-02
LysoPC(14:0)	22.1	1.17	2.95	5.26E-04	1.28E-03	0.88	1.68	5.28E-02	8.82E-02	1.24	0.57	8.80E-03	2.94E-02
Dodecanoic acid*	13.38	1.2	2.88	7.12E-05	2.59E-04	1.17	2.15	2.77E-03	7.91E-03				
Phosphate (3:1)*	8.73	1.16	2.87	2.72E-04	7.67E-04	1.2	1.51	1.64E-03	5.39E-03	1.34	0.53	2.92E-03	1.30E-02
Isoleucine*	8.95	1.22	2.74	2.26E-05	1.21E-04	0.78	1.59	9.50E-02	1.41E-01	1.19	0.58	1.37E-02	4.15E-02
Stearoyl-glycerol*	23.95	1.18	2.67	1.16E-04	3.78E-04	1.33	2.77	3.23E-05	3.29E-04				
Threonine*	10.26	1.15	2.67	4.23E-04	1.08E-03	0.63	1.59	1.89E-01	2.58E-01				
Serine*	9.88	1.07	2.51	2.53E-03	4.90E-03	0.4	1.28	4.22E-01	4.99E-01	1.16	0.51	1.78E-02	4.95E-02
Pantothenic acid	11.94	1.1	2.5	3.55E-03	6.66E-03	1	1.88	3.78E-02	6.91E-02				
Threitol*	11.87	1.05	2.47	3.82E-03	7.05E-03	0.42	1.34	4.04E-01	4.85E-01	1.23	0.54	1.04E-02	3.29E-02

Metabolite ^a	Retention time (min)	HF vs. Ctrl			LG vs. Ctrl			HF vs. LG					
		VIP ^b	FC ^c	p ^d	Adjusted p ^e	VIP ^b	FC ^f	p ^d	Adjusted p ^e	VIP ^b	FC ^g	p ^d	Adjusted p ^e
Leucine*	8.63	1.18	2.41	1.27E-04	3.92E-04	0.22	1.11	6.70E-01	7.21E-01	1.32	0.46	3.39E-03	1.44E-02
Benzoic acid, 4-hydroxy	13.16	1.1	2.39	1.41E-03	2.89E-03	0.63	1.56	1.93E-01	2.61E-01				
Valine*	7.83	1.2	2.13	7.14E-05	2.59E-04	0.52	1.38	2.87E-01	3.57E-01				
Cholesterol*	28.03	1.11	2.05	1.24E-03	2.58E-03	1.25	2.01	4.69E-04	2.28E-03				
Glutaric acid*	10.36	1.11	2.01	1.03E-03	2.21E-03	0.56	1.8	2.49E-01	3.14E-01				
5-Oxoproline*	11.96	1.13	1.98	6.77E-04	1.53E-03	0.37	1.13	4.60E-01	5.29E-01	1.48	0.57	1.42E-04	2.05E-03
2-Methylacetoacetic acid	3.63	1.19	1.71	4.36E-04	1.10E-03	0.89	0.73	7.53E-02	1.18E-01	1.48	0.43	1.52E-04	2.05E-03
Indoleacrylic acid	13.9	1.16	1.63	9.91E-04	2.14E-03	0.82	1.24	1.20E-01	1.69E-01	1.38	0.76	1.50E-03	7.82E-03
4-Hydroxyproline*	12.03	1.15	1.52	4.04E-04	1.04E-03	0.84	0.83	6.80E-02	1.09E-01	1.45	0.54	3.81E-04	3.63E-03
LysopC(16:0)	22.37	1.18	1.34	2.68E-04	7.65E-04	0.85	1.11	4.97E-02	8.49E-02	1.42	0.83	6.98E-04	5.04E-03
Betaine*	3.89	1.09	1.28	1.91E-03	3.83E-03	0.34	1.03	7.96E-01	8.31E-01				
Melatonin	20.44	1.2	0.7	9.48E-05	3.17E-04	1.35	0.6	1.34E-05	1.80E-04	1.43	0.86	6.31E-04	4.92E-03
Hydroxylamine	7.42	1.22	0.69	1.94E-05	1.12E-04	1.33	0.74	2.61E-05	2.79E-04				
Carnitine	6.44	1.23	0.61	1.26E-05	8.20E-05	1.33	0.66	3.55E-05	3.45E-04				
Urocanic acid*	4.06	1.23	0.45	4.01E-05	1.75E-04	1.32	0.57	1.44E-04	9.92E-04	1.17	1.27	1.69E-02	4.77E-02
Hydroxyacetic acid	5.73	1.26	0.4	2.22E-07	4.33E-06	1.39	0.31	4.66E-08	2.00E-06	1.33	0.78	3.25E-03	1.42E-02
Threonic acid *	12.52	1.19	0.33	7.58E-05	2.70E-04	0.56	1.26	2.48E-01	3.14E-01	1.38	3.79	1.43E-03	7.82E-03
Glycerol phosphate *	14.82	1.24	0.3	3.23E-06	2.88E-05	1.31	0.47	6.71E-05	5.53E-04	1.19	1.58	1.41E-02	4.18E-02
Ribofuranose	14.91	1.25	0.19	5.05E-07	7.38E-06	1.17	0.37	2.55E-03	7.50E-03				
Arabitol	14.42	1.18	0.17	1.26E-04	3.92E-04	1.25	0.27	5.23E-04	2.43E-03				
Uric acid*	18.52	1.22	0.16	2.19E-05	1.20E-04	1.37	0.03	1.73E-06	3.36E-05				
5-Methylcytidine	3.53	1.19	0.16	2.82E-04	7.84E-04	1.31	0.15	2.32E-04	1.42E-03				
Glucuronic acid	16.97	1.23	0.13	9.11E-06	6.72E-05	1.25	0.29	5.35E-04	2.43E-03				
Phenol*	7.29	1.28	0.11	6.52E-11	1.40E-08	1.4	0.12	5.40E-11	1.15E-08				
Histidine*	3.24	1.12	0.1	2.55E-03	4.90E-03	1.24	0.04	1.79E-03	5.56E-03	1.2	0.42	1.37E-02	4.15E-02
Methylguanidine*	3.66	1.11	0.1	2.98E-03	5.65E-03	1.21	0.13	3.88E-03	1.06E-02				
Arabinofuranose	13.01	1.24	0.08	5.07E-06	4.34E-05	1.34	0.15	1.88E-05	2.23E-04				
Glutamine*	14.85	1.24	0.03	2.68E-06	2.61E-05	1.3	0.4	9.95E-05	7.09E-04	1.59	14.63	5.07E-08	1.08E-05
Glucose*	16.41	1.26	0.01	1.33E-07	3.17E-06	1.28	0.39	2.33E-04	1.42E-03	1.39	83.13	1.24E-03	7.36E-03
Mannopyranose	17.2									1.19	32.16	1.38E-02	4.15E-02

Metabolite ^a	Retention time (min)	HF vs. Ctrl			LG vs. Ctrl			HF vs. LG		
		VIP ^b	FC ^c	p ^d	Adjusted p ^e	VIP ^b	FC ^c	p ^d	Adjusted p ^e	Adjusted p ^e
Glycerol*	8.69					1.17	2.54	1.67E-02	4.76E-02	4.76E-02
Oxalic acid	6.71					1.29	2.1	4.92E-03	1.85E-02	1.85E-02
Linoleic acid*	22.92					1.4	1.31	1.04E-03	6.48E-03	6.48E-03
2-Oxo-4-methylvaleric acid	7.77					1.18	1.25	1.50E-02	4.39E-02	4.39E-02
Tryptophan*	13.9					1.38	0.76	1.40E-03	7.82E-03	7.82E-03
Urea*	7.36					1.26	0.6	7.38E-03	2.55E-02	2.55E-02
Arachidonic acid*	21.17					1.52	0.39	3.93E-05	1.05E-03	1.05E-03
Glutamic acid*	13.12					1.26	0.28	7.20E-03	2.53E-02	2.53E-02
Ribose*	13.94					1.26	0.28	7.63E-03	2.59E-02	2.59E-02
4,7,10,13,16,19-Docosahexaenoic acid*	22.45					1.5	0.23	7.91E-05	1.69E-03	1.69E-03
Fructose*	16.09					1.42	0.17	7.30E-04	5.04E-03	5.04E-03
Glyceric acid*	9.48					1.23	0.11	1.02E-02	3.29E-02	3.29E-02

^a Asterisks (*) indicate metabolites are verified by reference standards.

^b Variable importance in the projection (VIP) was obtained from PLS-DA model with a threshold of 1.0.

^c Fold change (FC) was obtained by comparing the metabolites in the HF group to those of the Ctrl group.

^d p value was calculated from Student's *t* Test.

^e Adjusted p value was calculated with false discovery rate (FDR).

^f FC was obtained by comparing the metabolites in the LG group to those of the Ctrl group.

^g FC was obtained by comparing metabolites in the LG group to those of the Ctrl group. FC with a value >1 indicates a relatively higher concentration present in the HF group or the LG group while a value <1 means a relatively lower concentration as compared to the controls. FC with a value >1 also indicates a relatively higher concentration present in the LG group while a value <1 means a relatively lower concentration as compared to the HF group.

Table 2

The most significantly altered canonical pathways between HF and Ctrl revealed by IPA analysis.

Ingenity Canonical Pathways	p value	Differentially expressed metabolites
tRNA Charging	1.55E-09	L-alanine, L-tyrosine, L-proline, L-threonine, L-serine, L-leucine, L-isoleucine, L-valine, L-lysine, L-methionine, L-histidine, glycine, L-glutamine, L-phenylalanine
Glycine Betaine Degradation	7.59E-05	betaine, L-methionine, glycine, L-serine, pyruvic acid
Glycine Biosynthesis III	2.19E-04	L-alanine, glycine, pyruvic acid
L-serine Degradation	4.37E-03	L-serine, pyruvic acid
Pyruvate Fermentation to Lactate	8.51E-03	pyruvic acid, L-lactic acid
Alanine Degradation III	8.51E-03	L-alanine, pyruvic acid
Alanine Biosynthesis II	8.51E-03	L-alanine, pyruvic acid
Phosphatidylethanolamine Biosynthesis III	8.51E-03	L-serine, ethanolamine
Glycine Biosynthesis I	8.51E-03	glycine, L-serine
Tyrosine Biosynthesis IV	8.51E-03	L-tyrosine, L-phenylalanine
Methionine Salvage II (Mammalian)	2.04E-02	betaine, L-methionine
Uracil Degradation II (Reductive)	2.75E-02	uracil, beta-alanine
Threonine Degradation II	2.75E-02	glycine, L-threonine
Phenylalanine Degradation I (Aerobic)	2.75E-02	L-tyrosine, L-phenylalanine
Glutamate Receptor Signaling	2.75E-02	glycine, L-glutamine
Phenylalanine Degradation IV (Mammalian, via Side Chain)	3.02E-02	glycine, L-glutamine, L-phenylalanine
Folate Transformations I	3.47E-02	L-methionine, glycine, L-serine
Basal Cell Carcinoma Signaling	3.89E-02	cholesterol
Role of Lipids/Lipid Rafts in the Pathogenesis of Influenza	3.89E-02	cholesterol
Superpathway of Methionine Degradation	3.98E-02	betaine, L-methionine, L-serine, pyruvic acid
4-hydroxyproline Degradation I	4.47E-02	pyruvic acid, trans-4-hydroxy-L-proline
Histidine Degradation VI	4.47E-02	urocanic acid, L-histidine
dTMP De Novo Biosynthesis	4.47E-02	glycine, L-serine
Tyrosine Degradation I	4.47E-02	L-tyrosine, homogentisic acid