

Supplementary Materials and Methods

Cell culture

Colon cancer cell lines, HCT116 and LoVo, were purchased from ATCC. A normal colonic epithelial cell line, NCM460, was obtained from INCELL Corporation (San Antonio, TX) as control. All cells were grown in high-glucose Dulbecco's Modified Eagle's Medium (Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific), 2 mM L-glutamine, 50 U/ml penicillin and 50 µg/ml streptomycin in a humidified atmosphere containing 5% CO₂.

Culture supernatant of *L. gallinarum*

After culturing *L. gallinarum*, *L. casei* or *E. coli* MG1655 in BHI for 1-2 days, the bacterial concentration in each culture medium was measured using NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE). BHI cultures were diluted to ensure equal concentrations of *L. gallinarum*, *L. casei* and *E. coli*. Culture supernatants were then collected by centrifugation at 5,000 rpm for 10 minutes, followed by sterile filtration with a 0.22-µm membrane. Filtrates were collected and termed as *L. gallinarum* culture supernatant (LGCS), *L. casei* culture supernatant (LCCS) or *E. coli* MG1655 culture supernatant (ECCS).

Bacterial culture supernatant was separated into low-molecular-weight (LMW) and high-molecular-weight (HMW) using Amicon Ultra-15 Centrifugal Filter Units with a pore size of 3 kilodalton (kDa, Millipore, Bedford, MA). After centrifugation in a swinging bucket rotor at 4,000× g for 30 minutes, cells were cultured in medium

containing LGCS, ECCS or BHI (LMW, 10%; HMW, 1%). The bacterial supernatants were heated at 100°C for 30 minutes or treated with proteinase K (50 ug/ml; QIAGEN GmbH, Hilden, Germany). 10% heat-inactivated or proteinase K-treated bacterial culture supernatant was used for 3-(4,5-dimethylthiazoly-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Colony formation assay

Cells were seeded overnight on 12-well microplates at 5.0×10^2 , 1.0×10^3 and 3.0×10^3 cells per well. 20% of LGCS, ECCS or BHI broth was then added to the medium and cultured for 14 to 21 days. Cells were washed with phosphate-buffered saline and fixed in methanol, prior to staining with 0.5% crystal violet. Colonies were counted manually and relative colony formation was calculated using formula: relative colony formation (%) = (number of colony formed/average colony number in control group) \times 100%.

TUNEL staining assay

Paraffin-embedded colonic sections were used for TUNEL staining. The cell apoptosis index was determined by the total number of TUNEL positive cells in both tumor and normal tissues.

DNA extraction and 16S ribosomal DNA (rDNA) gene amplification from *Apc*^{Min/+} mouse stool samples after gavage of *L. gallinarium*

Apc^{Min/+} mouse stool samples were disrupted by bead-beating after digesting with mutanolysin (10 U/ul, Sigma-Aldrich) and lysozyme enzyme cocktail, as described in

our previous study¹³. DNA extraction and purification were performed using DNeasy PowerSoil kit (Qiagen, Hilden, Germany). Amplicon library for bidirectional (466 bp) sequencing on Illumina MiSeq platform was constructed using universal primers 341f, 5'-CCTAYGGGRBGCASCAG-3' and 806r, 5'-GGACTACNNGGGTATCTAAT-3' targeting across 16S rDNA genes V3-V4 hypervariable regions. Library clean-up and normalization was performed using the NEBNext Ultra DNA Library Pre kit (New England Biolabs, Ipswich, MA).

Metabolomics profiling for fecal samples

Fecal samples from each mouse were weighed (50 mg) for metabolomic profiling. Cold methanol (80%) was used for fecal metabolites extraction. The extracted samples were then centrifuged at 21,500× g for 15 min at 4°C, and the supernatant was subjected for LC-MS/MS analysis. MS raw files were processed using the same method as described above.

Targeted mass spectrometry

The standard solution of each standard substance was prepared by diluting the stock solution into a final concentration of 1 mmol/L. A stepwise dilution of this standard solution (internal standard was contained) was then prepared to obtain a series of calibration standard solutions. A total of 25 mg fecal samples from each mouse or 200 µL bacteria culture supernatant were used for tryptophan-related metabolites extraction. After the addition of 0.5mL extraction buffer (acetonitrile-methanol-water, 2:2:1. 0.1% formic acid and internal standard were contained), all the samples were homogenized at 35 Hz for 2-3 cycle and sonicated in an ice-water bath for 5 min. After

centrifugation at 21,500× g for 15 min at 4°C, the resulting supernatant was dried by vacuum freeze-drying and dissolved in 10% methanol for UHPLC-MS/MS analysis. SCIEX Analyst Work Station (Version 1.6.3) and Sciex MultiQuant (Version 3.0.3) were employed for MRM data acquisition and processing. Differential abundance analysis was performed with 2-tailed Mann-Whitney U test. Metabolites with adjusted P -value < 0.05 were considered statistically significant.