Biochemical and Biophysical Research Communications xxx (2016) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Short chain fatty acids induce UCP2-mediated autophagy in hepatic cells

Liliana F. Iannucci [a], Jingfeng Sun [b], Brijesh K. Singh [b], Jin Zhou [b], Vincent A. Kaddai [c], Antonia Lanni [a], Paul M. Yen [b, **], Rohit A. Sinha [b, *]

[a] Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Seconda Università degli Studi di Napoli, Caserta, Italy
[b] Program of Cardiovascular and Metabolic Disorders, Duke-NUS Medical School, 8 College Road, Singapore 169857, Singapore
[c] Baker IDI Heart and Diabetes Institute, Melbourne, Victoria 3004, Australia

ARTICLE INFO

Article history:
Received 18 October 2016
Accepted 19 October 2016
Available online xxx

Keywords:
SCFA
Microbiome
Autophagy
UCP2

ABSTRACT

Short-chain fatty acids (SCFAs) are gut microbial fermentation products derived from dietary fiber sources. Although depletion of gut microflora has been linked to the development of liver disease, the direct effects of SCFAs on intracellular hepatic processes are not well understood. In this study, we demonstrated that the SCFAs, propionate and butyrate, regulated autophagic flux in hepatic cells in a cell-autonomous manner. Induction of autophagy by SCFAs required PPARγ stimulation of Uncoupling Protein 2 (UCP2) expression that was associated with reduced intracellular ATP levels and activation of PRKAA1/AMPK (protein kinase, AMP-activated, alpha 1 catalytic subunit). In addition, elimination of gut flora by chronic antibiotic treatment diminished basal hepatic autophagy in mice suggesting that gut microbiota can regulate hepatic autophagy. These findings provide novel insights into the interplay between diet, gut microbiota, short chain fatty acids, and hepatic autophagic signaling.

1. Introduction

The gut microbiome represents a co-evolved symbiotic partnership that is found in many mammals, including humans. There are over a trillion microbial bacteria that thrive in our intestines and they can exert profound effects on host metabolism [1,2]. In recent years, there has been major interest in studying gut microbes and their metabolites since they may play central roles in the pathogenesis of metabolic diseases [3]. Not surprisingly, microbial dysbiosis also has been linked to several diseases in the liver [4–9]. One of the primary mechanisms by which the gut microbiome influences host metabolism is the generation of short-chain fatty acids (SCFAs) such as acetate, butyrate, and propionate from anaerobic fermentation of dietary fibers [10]. Besides serving as a major fuel source for the intestinal epithelial cells, SCFAs also can enter the entero-hepatic circulation and contribute to hepatic lipogenesis and gluconeogenesis [11]. Of note, the transfer of intestinal microbiota from lean donors to individuals with metabolic syndrome led to improvement in insulin sensitivity and increased the number of intestinal butyrate-producing microbiota and fecal SCFAs [12–14]. Although it is known that diets rich in fibers can be beneficial for health and metabolism, the direct effects of SCFAs on the liver are poorly understood [15–19].

Macroautophagy (hereafter referred as autophagy) is an indispensable cellular process that can be dysregulated in liver diseases involving lipid or carbohydrate imbalances [20–22]. The induction of autophagy has been reported to have beneficial effects in several liver diseases [23]. Previously, SCFAs have been linked to autophagy regulation in colon cancers [24]; however, their action(s) on autophagy in hepatic cells is not known.

In this study, we assessed the effect of SCFAs, propionate and butyrate, on hepatic autophagy in a cell-autonomous manner. Our results revealed that SCFAs induce autophagic flux in mouse hepatic cells in a UCP2-AMPK dependent manner. Moreover, our results showed that the loss of gut microbiota after antibiotic

Abbreviations: ACTB, actin beta; ATP, adenosine triphosphate; MAP1LC3B, microtubule-associated protein 1 light chain 3B; PRKAA1, protein kinase, AMP-activated, alpha 1 catalytic subunit; SCFA, short-chain fatty acids; PPAR-γ, peroxisome proliferator-activated receptor gamma; TCA, tricarboxylic acid; UCP2, uncoupling protein 2.

* Corresponding author. Cardiovascular and Metabolic Disorders Program, Duke-NUS Medical School, 8 College Road, Level 8, Singapore 169857, Singapore.
** Corresponding author. Cardiovascular and Metabolic Disorders Program, Duke-NUS Medical School, 8 College Road, Level 8, Singapore 169857, Singapore.
E-mail addresses: paul.yen@duke-nus.edu.sg (P.M. Yen), rohit.sinha@duke-nus.edu.sg, anthony.rohit@gmail.com (R.A. Sinha).

http://dx.doi.org/10.1016/j.bbrc.2016.10.072
0006-291X/

treatment impaired basal hepatic autophagy. Taken together, these results provide a novel insight into the role of SCFAs and gut microbiota in host liver physiology.

2. Materials and methods

2.1. Reagents

Antibody details are as follows: LC3B (Cell Signaling Technology, 2775), PRKAA1/AMPKα1 (Cell Signaling Technology, 2795); phospho-PRKAA1/AMPKα (Cell Signaling Technology, 4188), GAPDH (Cell Signaling Technology, 5174), ACTB/β-Actin (Santa Cruz Biotechnology, sc-81178), UCP2 (Santa Cruz Biotechnology, sc-6525). Culture media and transfection reagents were from Invitrogen, USA. siRNA used are as follows: *Ucp2 (*Ambion®, 4390771). Butyric acid (SIGMA-ALDRICH, B103500), Propionic acid (SIGMA-ALDRICH, P1386), Ampicillin (SIGMA-ALDRICH, A9518) and Neomycin (SIGMA-ALDRICH,N1876). ptfLC3 (Plasmid #21074) was from Addgene.

2.2. Cell culture

AML-12 (CRL-2254) cells were maintained at 37 °C in DMEM-F12 1:1 containing 10% fetal bovine serum, 1× ITS (ThermoFisher Scientific, 41400), 10 nM dexamethasone and 1× penicillin/streptomycin in a 5% CO_2 atmosphere. For siRNA transfection, cells were transfected using RNAiMAX (Thermo Fisher Scientific, 13778) with either specific siRNA's or negative siRNA's, and for overexpression experiments, Lipofectamine 3000 (Thermo Fisher Scientific, L3000001) with gene specific or empty plasmids, following the manufacturer's reverse-transfection protocol. For knockdown studies, cells were treated with specific siRNA (10 nM) for 48 h before adding SCFA. Negative siRNA (Silencer® Negative Control No. 1 siRNA, AM4611; Life Technologies Inc.) was also used in parallel as negative control.

2.3. RNA isolation and real-time PCR

Total RNA was isolated and qPCR performed using the Quanti-Tect SYBR Green PCR Kit (Qiagen, 204141) in accordance with the manufacturer's instructions. Primer sequence were *Ucp2*: 5′-ACCTTTAGAGAAGCTTGACC-3′ (sense) 5′-TTCTGATTTCCTGC-TACCTC-3′ (antisense), RNApolII 5′-CGGTTGAATCTTAGTGTGAC -3′ (sense) 5′-ATAGCCAACTCTTGGATCTC-3′ (antisense).

2.4. Western blotting

Cells or tissue samples were lysed using CelLytic™ M Cell Lysis Reagent (Sigma, C2978) and immunoblotting was performed as described previously [22]. Image acquisition was done using ChemiDoc (Bio-Rad ChemiDoc™ MP System, 1708280). Densitometry analysis was performed using ImageJ software (NIH, Bethesda, MD, USA).

2.5. Immunofluorescence studies

Immunofluorescences experiments were performed as described previously [22]. In brief formalin-fixed cells were permeabilized with 0.1% Triton X-100 (SIGMA-ALDRICH, X100) in PBS for 5–10 min and blocked with 3% BSA-PBS for 30 min at room temperature. Cells were incubated with the primary antibody (1:200 in 3% BSA-PBS) overnight at 4 °C followed by incubation with an Alexa 488 labelled secondary antibody. Cell imaging was performed using a confocal microscope LSM710 Carl Zeiss (Carl Zeiss Microscopy GmbH, Germany). For Acridine orange staining cells were incubated with either 1 μg/mL of AO (Sigma-Aldrich) in PBS for 30 min at 37 °C, followed by three washes with PBS, and then immediately observed under a fluorescence microscope (Leica Microsystems Inc.) and captures using Leica application suite (LAS) software.

2.6. Seahorse XF-24 metabolic flux analysis

Oxygen consumption was measured at 37 °C using an XF24 extracellular analyzer (Seahorse Bioscience,USA). 30,000 AML-12 cells were seeded in 24-well XF24 culture plates and treated with or without T_3 prior to the assay. Optimization of reagents was performed using the Mito stress test kit from Seahorse Bioscience (103015-100) using the protocol and algorithm program in the XF24 analyzer.

2.7. Intracellular ATP measurement

Intracellular ATP was measured following manufacturer's guidelines (ABCAM, ab113849).

2.8. Metabolomics analysis of organic acids

Cells were homogenized in 50% acetonitrile, 0.3% formic acid. Data acquisition and analysis were performed on Agilent Mass-Hunter Workstation B.06.00 Software. Trimethylsilyl derivatives of organic acids were separated by gas chromatography on an Agilent Technologies HP 7890A and quantified by selected ion monitoring on a 5975C mass spectrometer using stable isotope dilution. The initial GC oven temperature was set at 70 °C, and ramped to 300 °C at a rate of 40 °C/min, and held for 2 min.

2.9. Animals and antibiotic treatment

Male C57BL/6 mice (8- to 10-weeks old) were purchased and housed in hanging polycarbonate cages under a 12 h/12 h light/dark schedule. Animals were euthanized in CO_2 chambers, and blood was drawn by cardiac puncture. All mice were maintained according to the Guide for the Care and Use of Laboratory Animals (NIH publication no. 1.0.0. Revised 2011), and experiments were approved by the IACUCs at Duke-NUS Graduate Medical School. The oral antibiotics were administered in the drinking water for 13 weeks in the following concentrations: ampicillin (1.0 g/L) and neomycin (0.5 g/L). The antibiotics were dissolved in water and filtered with 0.22 μm syringe filters before being added to drinking water. The bottles and water were changed every three days as ampicillin is unstable in water at neutral pH. The mice were necropsied after 12 weeks of treatment and the mouse liver was extracted.

2.10. Bacterial DNA extraction from fecal samples

QIAampR DNA Stool Mini Kit, were used to lyse bacterial cells followed by extraction of chromosomal DNA from cell lysates. QIAampR DNA Stool Mini Kit was purchased from (QIAGEN, Cat No./ID: 51504) Canada and used according to the manufacturer's instructions.

2.11. Calculations and statistics

Results were expressed as mean ± SD. The statistical significance of differences ($^*P < 0.05$) was assessed by ANOVA analyses followed by Tukey's post-hoc when comparing different groups.

Please cite this article in press as: L.F. Iannucci, et al., Short chain fatty acids induce UCP2-mediated autophagy in hepatic cells, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.10.072