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Light exposure influences the diurnal oscillation of gut microbiota in mice

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ABSTRACT

The gut microbiota exhibit diurnal compositional and functional oscillations that influence the host homeostasis. However, the upstream factors that affect the microbial oscillations remain elusive. Here, we focused on the potential impact of light exposure, the main factor that affects the host circadian oscillation, on the diurnal oscillations of intestinal microflora to explore the upstream factor that governs the fluctuations of the gut microbes. The gut microbiota of the mice that were underwent regular light/dark (LD) cycles exhibited a robust rhythm at both compositional and functional level, in all parts of the intestine. Comparably, constant darkness (Dark-Dark, DD) led to the loss of the rhythmic oscillations in almost all parts of the intestine. Additionally, the abundance of *Clostridia* in DD conditions was dramatically enhanced in the small intestine. Our data indicated light exposure is the upstream factor that governs the regular diurnal fluctuations of gut microbiota *in vivo*.

1. Introduction

In response to the fluctuations of light caused by the rotation of the earth, all the domains of life including prokaryotic and eukaryotic organisms, develop diverse molecular clock machinery [1–3]. The mammalian circadian clock comprises both a central clock system, which is located within the suprachiasmatic nucleus of the brain and a peripheral clock system, which is located in the peripheral organs, like the liver, heart and kidney [4–6], for example. The central clock system is entrained by environmental light/dark (LD) cycles [7]. Accordingly, impaired light exposure like constant darkness (DD), has been shown to lead to the disruption of rhythmic oscillations of circadian parameters [8–10]. Therefore, light is the main environmental factor that influences the circadian clock.

Recently, increasing attention has been paid to the rhythmic oscillations of the gut microbiota. Studies have demonstrated that the gut microbiota exhibit compositional and functional fluctuations [11,12]. Consequently, disruption of these oscillations, such as host clock genes depletion and a high-fat diet [13,14], would impair host homeostasis and facilitate disease development [11,15,16]. Although the downstream effects of gut microbial arrhythmic oscillations are becoming evident, the upstream environmental factors that govern the gut microbial oscillations remain unknown. In the present study, we systematically explored the impact of the main environmental factor, light, on the diurnal oscillation of gut microbiota located in the lumen, mucus layer and epithelial layer, regarding both compositional and functional outcomes.

2. Materials and methods

2.1. Animals

Male 5 week-old Balb/c mice were used in the present study. All mice were randomized into two groups: light-dark (LD) group and dark-dark (DD) group. The LD animals were strictly maintained in 12 h/12 h light-dark cycles with light on at 8:00 a.m. and off at 8:00

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p.m. (ZT0 = 8 a.m.), the LD group was fed a standard diet ad libitum during both light and dark phase. The DD group was housed in a dark condition and fed a standard diet ad libitum during the whole experiment. All animals were anesthetized and sacrificed after two weeks treatment, samples were collected every 4 h starting at ZT2 (n = 4–5 mice for each time point). For the tissue harvesting during night or dark phase, mice were anesthetized first in the dark and removed into light environment for samples collection. All animals care and study protocols were in accordance with the guidelines of the Institutional Animal Care and Use Committee at Southern Medical University.

2.2. DNA extraction

DNA from intestinal lumen, mucous layer, epithelial layer, and cecal contents were extracted as previously described [17,18]. Briefly, luminal contents were collected by flushing with 1 ml sterile phosphate-buffered saline (PBS). Then the intestine was cut longitudinally and washed in 1 ml PBS vigorously to collect the mucus layer associated microbe. Finally the remaining part of the intestine was homogenized in sterile PBS and used for epithelial layer associated bacteria isolation. Intestinal contents, mucous layer and epithelial layer were resuspended separately in PBS containing 0.5% Tween 20 and then placed to a −80 °C/60 °C cycle three times to destroy the membrane. DNA extraction was performed manually as the phenol-chloroform method.

2.3. Microbiota analysis

For 16S amplicon sequencing, PCR amplification was performed spanning the bacterial V4-16S rRNA and the barcodes (primers used for V4-16s amplification) were added to the samples to facilitate sequencing by qPCR, the primers were described in the Supplementary data (Table S1), the PCR products were mixed at a certain ratio by Qubit fluorometer (InvitrogenTM). The Illumina Hiseq PE250 sequencing platform was used for further sequencing. 16S rRNA reads were initially screened for low quality bases and short read lengths. Paired-end read pairs were then assembled using SeqPrep and the resulting consensus sequences were demultiplexed (i.e., assigned to their original sample), trimmed of artificial barcodes and primers, and assessed for chimeras using UCHIME in closed mode implemented in Quantitative Insights Into Microbial Ecology (QIIME; release v. 1.9.1). Quality trimmed sequences were then clustered closed into Operational Taxonomic Units (OTUs) by SortMeRNA (v2.0) with GreenGene's database (v13_8) in QIIME, with a minimum confidence threshold of 0.97 for the taxonomic assignments (sharing 97% similarity). The SortMeRNA (v2.0) was used to classify these sequences into specific taxa using the GreenGene's database (v13_8). Multiple alignments were performed using PyNAST (1.2.2); the GreenGene's core set (version: 13.8) was used as the template file. To account for uneven sampling depth, the data were also rarefied to the minimum sampling depth of 1085 sequences. The observed otus were applied to evaluate alpha diversiaty. Estimation of the alpha diversity was performed with the Shannon and Chao metrics. Beta diversity was calculated by QIIME using UniFrac distance, Principal Coordinates Analyses (PCoA) plots was based on distance matrix from a weighted UniFrac phylogenetic method. And it was performed and visualized with R (v3.2.2) and ade4 R packages, then tested for significance with Adonis (999 permutations).

2.4. Functional pathways prediction analysis

PICRUSt (Phylogenetic Investigation of Communities by Reconstruction) software was used to impute the prevalence of 16S rRNA marker gene sequences in the difference in each group [19,20], the main oscillatory functional pathways were plotted in a heatmap using R software and pheatmap. Heatmap was represented the oscillating in the main function KEGG pathways in the class level, the values were centered and scaled in the row direction.

2.5. Statistical analysis

Data are presented as mean ± SEM, statistical analyses were performed using GraphPad Prism and R software. PCoA was implemented using the “ade4” and “vegan” package, significance for PCoA analyses was checked with “Adonis” (999 permutations). Kruskal Wailles test was applied to analyze the data at six time point (ZT2 − ZT22) in each group. Significance was evaluated using the unpaired Student's t-test with FDR correction, and the significance level was set to 0.05.

3. Results

3.1. Cecal microbe exhibited compositional and functional diurnal patterns that were influenced by light

We firstly monitored the day-night variations of the cecal microbes in LD mice. As presented in Fig. 1A, based on 16s RNA sequencing, principal coordinates analysis (PCoA) of weighted beta diversity, revealed separate plots for each time point cluster. According to PCoA results, the distance between each time point in LD group was significantly different ($p < 0.05$, Adonis analysis). Specifically, the PC1 distance (served as an important parameter for compositional comparison) of each time point from zeitgeber time 2 (ZT2) −ZT22, exhibited a robust rhythmic oscillation ($p < 0.05$, Kruskal Wailles test). However, the oscillation was completely lost by DD, as indicated from the Adonis analysis ($p > 0.05$) and Kruskal Wailles test ($p > 0.05$), suggesting the diurnal fluctuation of microbial beta-diversity was dependent on a L/D cycle. We next focused on specific strains. Representatively, similar to the PC1 distance, the relative abundance of *Bacteroidia* exhibited diurnal fluctuation in the LD group ($p < 0.05$, Kruskal Wailles test), while no fluctuation was observed in DD animals ($p > 0.05$, Kruskal Wailles test) (Fig. 1B). Besides, the abundance of *Bacilli* in the cecum, was dramatically elevated at ZT10 and ZT22 in LD, demonstrating these bacteria exhibited diurnal variation in the intestine ($p < 0.05$, Kruskal Wailles test). However, DD treatment abolished this variation ($p > 0.05$, Kruskal Wailles test) (Fig. 1B). In addition to the changes at the compositional level, we evaluated the oscillation of cecal microbes at the functional level, based on Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) analysis. We grouped all genes into KEGG pathways and selected the main pathways, as presented in Fig. 1C. In the LD group, the pathways of “Two-component system” and “Bacterial motility proteins” exhibited day/night fluctuation of microbiota functionality ($p < 0.05$, Kruskal Wailles test). However, the “amplitude” of the fluctuation was attenuated in DD group, only the pathways of “Oxidative phosphorylation” exhibited fluctuation ($p < 0.05$, Kruskal Wailles test). Collectively, these data demonstrated the diurnal oscillation of cecal microbial composition and function was influenced by light exposure.

3.2. The impact of light exposure on the diurnal pattern of intestinal bacteria in the lumen, mucus layer and epithelial layer

Beside the cecum, the intestinal microbiota located in the lumen, mucus layer and epithelial layer also play an important role in maintaining gut homeostasis [21,22] and exhibit diurnal oscillations. Thus, we next determined whether light would be an

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