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### **ORIGINAL ARTICLE**

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# Liver-specific knockdown of ANGPTL8 alters the structure of the gut microbiota in mice



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### Abstract

**Purpose:** To investigate the effect of liver-specific knockdown of ANGPTL8 on the structure of the gut microbiota.

**Methods:** We constructed mice with liver-specific ANGPTL8 knockdown by using an adeno-associated virus serotype 8 (AAV8) system harbouring an ANGPTL8 shRNA. We analysed the structure and function of the gut microbiome through pyrosequencing and KEGG (Kyoto Encyclopedia of Genes and Genomes) functional prediction.

**Results:** Compared with controls, ANGPTL8 shRNA reduced the Simpson index and Shannon index (p < 0.01) of the gut microbiota in mice. At the phylum level, the sh-ANGPTL8 group showed a healthier gut microbiota composition than controls (*Bacteroidetes*: controls 67.52%, sh-ANGPTL8 80.75%; *Firmicutes*: controls 10.96%, sh-ANGP TL8 8.58%; *Proteobacteria*: controls 9.29%, sh-ANGPTL8 0.98%; F/B ratio: controls 0.16, sh-ANGPTL8 0.11). PCoA and UPGMA analysis revealed a significant difference in microbiota composition, while KEGG analysis revealed a significant difference in microbiota group.

**Conclusion:** Our results revealed that inhibition of ANGPTL8 signalling altered the structure of the gut microbiome, which might further affect the metabolism of mice. We have thus identified ANGPTL8 as a novel hepatogenic hormone potentially involving the liver-gut axis and regulating the structure of the gut microbiota.

Keywords: ANGPTL8, Gut microbiota, Pyrosequencing, KEGG functional prediction

### Introduction

Incredible amounts of microorganisms exist in the human intestinal tract, the population of which reaches 100 trillion, far more than other microorganisms on the human surface, approximately 10 times the sum of both somatic cells and germ cells (Backhed et al. 2005). There are multifarious factors, especially those related to diet, involved in the regulation of the gut microbiota. It has been reported that specific diets or nutrients are beneficial or deleterious to the equilibrium of the gut microbiota. For instance, prebiotic, resistant starch and dietary polyphenols revealed the capacity to improve the host's metabolic status and reduce the risks of chronic diseases, while high-fat diet (HFD) exacerbated dysbiosis of the gut microbiota and was involved in inflammatory processes (Sanchez-Tapia et al. 2019; Singh et al. 2018; Gowd et al. 2019). Diet is a crucial regulator towards the gut microbiota, though it is not the only regulator. The gastrointestinal (GI) tract, the largest endocrine organ, has been reported to secrete more than 20 different hormones, such as peptide YY (PYY) and glucagon-like peptide 1 (GLP-1) (Mishra et al. 2016). Recent studies showed that pre-menopausal women revealed a higher F/B (Firmicutes to Bacteroidetes) ratio, an elevated relative abundance of Roseburia and Lachnospira, as well as elevated GLP-1 plasma levels compared with postmenopausal women (Santos-Marcos et al. 2018). Sex steroid hormone levels, including serum levels of



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testosterone in men and oestradiol in women, were associated with the diversity and composition of the gut microbiota (Shin et al. 2019). In addition, treatment with progesterone in ovariectomized (OVX) mice has been reported to change the gut microbiota composition significantly (Sovijit et al. 2019). It is noteworthy that the GI tract is unable to secrete sex hormones, which indicates that the gut microbiota potentially respond to the hormones secreted from remote organs.

Various chronic diseases are associated with the dysbiosis of gut microbiota, such as obesity, cancer, inflammatory bowel disease and autism (Zhang et al. 2015). Chronic liver disease (CLD) has attracted extensive attention, as the liver is the largest metabolic organ in the human body. In addition, from the perspective of physiology, the liver is the most important extra-intestinal organ that has crosstalk with the intestine through the gut-liver axis (Konturek et al. 2018). As microbial products and gut-derived toxins can enter hepatocytes through the portal vein, the abnormal composition changes the gut microbiota, consequently contributing to CLD, especially non-alcoholic fatty liver disease (NAFLD) and hepatic encephalopathy (Minemura and Shimizu 2015). However, previous studies have shown that the liver tissue influences the structure of the gut microbiota through secreting bile acids. Abnormal secretion of bile acids plays an important role in the dysbiosis of gut microbiota (Quesada-Vazquez et al. 2020).

ANGPTL8, also known as betatrophin, lipasin and C19orf80, belongs to the angiogenin-like protein family. The angiogenin-like protein family includes eight members structurally similar to angiogenin and widely participate in the regulation of lipid metabolism, inflammation, haematopoietic stem cell activity and cancer cell invasion. ANGPTL8 is highly enriched in the human liver and various adipose tissues, whose levels were much lower in the other tissues, and recent studies suggested that ANGPTL8 showed similar tissue distribution in mice (Chen et al. 2019; von Loeffelholz et al. 2017; Catalano-Iniesta et al. 2020; Akimoto et al. 2019; Mysore et al. 2017). ANGPTL8 has been reported to play a significant role in various metabolic diseases. For instance, the serum level of ANGPTL8 was increased in diabetic patients and was related to serum levels of total cholesterol (TC), low-density lipoprotein (LDL) and apolipoprotein B. HFD induced increased transcriptional levels of ANGPTL8 in the mouse liver, brown adipose tissue (BAT) and white adipose tissue (WAT). Consistently, serum levels of triglyceride (TG) increased when ANGP TL8 was overexpressed by adenovirus infection (Chen et al. 2019; Siddiqa et al. 2017).

Although there is enough convincing evidence illustrating that ANGPTL8 is associated with diseases involving multiple systems, the relationship between ANGP TL8 and the gut microbiome is still unclear. Notably, only ANGPTL4 has been found that has a strong association with the gut microbiota in the angiogenin-like protein family. Upon treating the colon carcinoma cell line HCT116 with Lactobacillus paracasei spp. paracasei F19, Bifidobacterium lactis 12 and Lactobacillus rhamnosus GG, the level of ANGPTL4 was upregulated (Aronsson et al. 2010). It is worth mentioning that unlike ANGPTL8, ANGPTL4 is able to be produced by entero-endocrine cells (EEC) in human intestine (Alex et al. 2014). However, the relationship between ANGP TL8 and the gut microbiota is unclear. To explore the role of ANGPLT8 (a hepatokine) and its potential regulatory effects on the gut microbiota in the liver-gut axis, we knocked down endogenous ANGPTL8 in the liver and investigated the influences towards gut microbiota homeostasis based on the next-generation sequencing. As ANGPTL8 mainly expresses in the liver, we constructed liver-specific ANGPTL8 knockdown mice using the adeno-associated virus serotype 8 (AAV8) system and performed pyrosequencing on 16S rDNA of the faecal microbiota genome. Herein, our results revealed that liver-specific knockdown of ANGPTL8 was able to alter the diversity, composition and functional pathways of the gut microbiome. As a result, the hepatogenic hormone, ANGPTL8, showed its ability to regulate the gut microbiota, which is another regulatory function from liver towards gut microbiota in addition to the only well-established enterohepatic circulation of bile acid. In addition, our results demonstrated a rare case of a gut microbiota response to non-enterogenic hormones except for sex hormones.

### **Materials and methods**

### Study design and animals

All animal procedures in this investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996) and were approved by the Laboratory Animal Care & Use Committee at China Pharmaceutical University (Permit number SYXK-2016-0011). Male C57BL/6J mice were maintained in a 12 h:12 h LD cycle in a temperature- and humidity-controlled environment. Mice were transduced with an AAV8 system carrying shRNA against scramble (as a negative control) (controls) or ANGPTL8 (sh-ANGPTL8) (designed and synthesised by Hanbio, Shanghai, China) into mice at a dose of  $1 \times 10^{12}$  vg for 45 days to knock down ANGPTL8 expression in the liver through tail-vein injection. After that, the mice were subjected to 12-h food deprivation and then were humanely killed to collect faeces. The following shRNA oligonucleotide sequences (5'-3') were used: Scramble shRNA: GTTCTCCGAACGTGTCACGTAATTCAAG

### AGATTACGTGACACGTTCGGAGAATTTTTT and ANGPTL8 shRNA: GTATGAAGAGCTGACCCTGCTC TTTCTTCAAGAGAGAAAAGAGCAGGGTCAGCTCTT CATATTTTT.

### Quantitative real-time PCR

Total RNA from mouse liver samples was isolated using TRIzol reagent (Invitrogen, Carlsbad, California, USA), reverse transcribed with the PrimeScript RT reagent kit (Takara, Tokyo, Japan) and analysed by real-time quantitative PCR using 2 × ChamQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China) according to the manufacturer's instructions. The relative expression levels were calculated using the  $2^{-\Delta\Delta Ct}$  method. Primers for mouse  $\beta$ -actin were used as an internal control. Detailed primer sequences are provided in Supplemental Table 1.

### Faecal DNA extraction and pyrosequencing

Faeces samples were collected in an aseptic environment from each of the 14 sacrificed mice, immediately snapfrozen in liquid nitrogen and stored at – 80 °C. DNA was extracted using a faecal DNA isolation kit (Qiagen, Germantown, MD, USA). The V4 regions of 16S rDNA genes were amplified using extracted DNA from each sample as template for later pyrosequencing performed by Novogene (Beijing, China), as described previously (Guo et al. 2016).

### Sequencing data processing

Single-end reads were assigned to samples according to their unique barcode and were truncated by trimming the barcode and primer sequence. Quality filtering on the raw reads was performed under specific filtering conditions to obtain high-quality clean reads based on the Cutadapt (Martin 2011) (V1.9.1, http://cutadapt. readthedocs.io/en/stable/) quality-controlled process. The reads were compared with the reference database (Silva database, https://www.arb-silva.de/) (Quast et al. 2013) through the UCHIME algorithm (UCHIME Algorithm, http://www.drive5.com/usearch/manual/uchime\_ algo.html) (Edgar et al. 2011) to detect chimaera sequences. Clean reads were ultimately obtained by removing the chimaera sequences (Haas et al. 2011).

### OTU cluster and species annotation

Sequence analysis was performed using Uparse software (Uparse v7.0.1001, http://drive5.com/uparse/) (Edgar 2013). Sequences with  $\geq$  97% similarity were assigned as the same OTUs. Representative sequences for each OTU were screened for further annotation. For each representative sequence, the Silva Database (https://www.arb-silva.de/) (Quast et al. 2013) was used through the Mothur algorithm to annotate taxonomic information. Multiple sequence alignments

were conducted using MUSCLE software (Version 3.8.31, http://www.drive5.com/muscle/) (Edgar 2004) to study the phylogenetic relationships of different OTUs, as well as the differences of the dominant species in different samples (groups). OTU abundance information was normalised based on a standard of sequence numbers corresponding to the sample with the least sequences. Subsequent analyses of alpha diversity and beta diversity were all performed using this output-normalised data.

### Alpha diversity

Alpha diversity was applied to analyse the complexity of species diversity for a sample based on 4 indices, including observed-species, Chao1, Simpson, and Shannon. All these indices in our samples were calculated with QIIME (Version 1.9.1) and were displayed with R software (Version 2.15.3). The difference of each index between groups was tested using the Wilcoxon rank-sum test.

### Beta diversity

Beta diversity based on Bray-Curtis distances was calculated using QIIME software (Version 1.9.1) to evaluate differences between samples in species complexity. Cluster analysis was preceded by the principal coordinate analysis (PCoA) to reduce the dimension of the original variables. PCoA analysis was displayed by the WGCNA package, stat packages and ggplot2 package in R software (Version 2.15.3). Unweighted Pair-group Method with Arithmetic Means (UPGMA) Clustering was performed as a type of hierarchical clustering method to interpret the distance matrix using average linkage based on QIIME software (Version 1.9.1). The Metastats analysis was performed as a statistical method for detecting species with significant differences among microbial communities in a group. p values were obtained by hypothesis testing of species abundance data between groups at the phylum level, while Q values were obtained by correcting p values. Species with significant differences were screened based on p or q values. Adonis analysis was performed by using the adonis function in the R vegan package. Manhattan plot was performed by arranging OTUs according to their taxonomy.

### **Functional prediction**

Tax4Fun functional prediction was achieved by the nearest neighbour method based on the minimum 16S rDNA sequence similarity. A correlation matrix was established by extracting the KEGG (Kyoto Encyclopedia of Genes and Genomes) database prokaryotic whole genome 16S rDNA gene sequence and aligning it to the SILVA SSU Ref NR database based on the BLASTN algorithm (BLAST Bitscore > 1500). The SILVA database functional annotation was implemented through mapping the prokaryotic whole genome functional information of the KEGG database annotated by UProC and PAUDA to the SILVA database. The functional annotation information was obtained by clustering sequenced samples out of the OTU based on the SILVA database sequence as a reference sequence.

### Statistical analysis

Data are presented as the means  $\pm$  SD. The data were analysed by one-way ANOVA followed by Fisher's LSD post hoc test. The 16S rRNA gene copies were normalised by log<sub>10</sub> transformation before statistical analysis. The richness estimators and the composition of the dominant bacterial genera were also analysed by either one-way ANOVA followed by Fisher's LSD post hoc test or Tukey's HSD test, when indicated. Calculations were performed by Origin8 8.6. p < 0.05 was considered statistically significant.

### Results

## Liver-specific knockdown of ANGPTL8 regulated the species diversity of gut microbiota through influencing the species evenness

First, we constructed mice with liver-specific Angptl8 knockdown by using an AAV8 system carrying an Angptl8 shRNA. Our previous research showed that AAV8-mediated knockdown of ANGPLT8 blocked its expression and secretion (Chen et al. 2019). In addition, as shown in Fig. 1a, the hepatic expression of ANGPTL8 was successfully blocked. To further explore the effects of ANGPTL8 on the gut microbiota, we analysed the bacterial 16S rDNA (V4 region) in mouse faeces through pyrosequencing-based analysis. After removing unqualified sequences, we obtained a total of 1,164,413 raw reads and an average of 83,172 ± 2457 reads per sample. After selecting the clean reads, we generated a total of 1, 126,981 clean reads, while each faecal sample (n = 7 per group) revealed an average of  $80,499 \pm 2412$  effective reads. Rarefaction analysis revealed that the sequencing



depth was able to cover almost all the diversity and rare new phylotypes (Fig. 1b). Comparing faeces samples of controls and sh-ANGPTL8, we observed no statistically significant differences in observed species and Chao1 index (p > 0.05) (Fig. 1c, d), which indicated that in the gut microbiota, there were no statistically significant differences in species richness. Conversely, Simpson index and Shannon index analyses showed statistically significant differences in species diversity (p < 0.01) (Fig. 1e, f). In consideration of the species diversity based on Simpson index and Shannon index, which includes the species richness and evenness, we suggested that liverspecific knockdown of ANGPTL8 regulated the species diversity of the gut microbiota through influencing the species evenness.

### Liver-specific knockdown of ANGPTL8 regulated the structure of the gut microbiota

PCoA based on Bray-Curtis distances revealed unique clustering of microbiota composition for the control and treatment groups (Fig. 2a). The first and second axes explained 91.72% and 4.67% of the total variance, with a significant *p* value (Adonis, *p* value = 0.001,  $R^2$  = 0.9174) (Fig. 2a). UPGMA analysis revealed a significant separation in the gut microbiota sample between controls and the sh-ANGPTL8 group, indicating that liver-specific ANGPTL8 knockdown altered the structure of the gut microbiota (Fig. 2b).

### Liver-specific knockdown of ANGPTL8 regulated the abundance of individual species

At the phylum level, we observed a significant bacterial composition difference between gut microbiota of the control and sh-ANGPTL8 groups (Supplemental Table 2). Most of the gut microbiota in the faecal samples of the control group consisted of Bacteroidetes (67.52%), Firmicutes (10.96%) and Proteobacteria (9.29%), while the abundances of the sh-ANGPTL8 group were represented by Bacteroidetes (80.75%), Firmicutes (8.58%) and Proteobacteria (0.98%) (Fig. 3a). Comparing the faecal samples of controls and sh-ANGPTL8 mice, we observed that the abundance levels of Bacteroidetes and Deferribacteres increased, while those of Firmicutes, Proteobacteria, Verrucomicrobia, Melainabacteria, Tenericutes, Actinobacteria and Acidobacteria decreased in the faecal samples of the sh-ANGPTL8 group in contrast to the control group, with a significant difference  $(p \leq p)$ 0.001) (Fig. 3b, c). Our results also revealed that the liver-specific knockdown of ANGPTL8 reduced the F/B (Firmicutes/Bacteroidetes) ratio (from 0.16 to 0.11) (Fig. 3f) because of the increased relative abundance of Bacteroidetes (Fig. 3d) and the decreased relative abundance of Firmicutes (Fig. 3e), indicating that the alteration of gut microbiota composition was conducive to impeding the collection of energy and decreasing the levels of chronic inflammation. It is noteworthy that although the relative abundance of Proteobacteria was not the highest in the gut microbiota, there was a dramatic reduction







(from 9.29 to 0.98%) in the sh-ANGPTL8 group (Fig. 3g), which markedly decreased the levels of chronic inflammation. The observed gut bacterial community shifts were dissected by arranging OTUs according to their taxonomy, and their enrichment was displayed in a set of Manhattan plots (see the "Materials and methods" section). Based on the Manhattan plots, we observed that almost all the OTUs with high abundance revealed significant differences between the control and sh-ANGPTL8 groups (Fig. 3h). Conversely, most of the ANGPTL8 OTUs that obviously shifted after

knockdown belonged to *Bacteroidetes* and *Firmicutes*, while few of the OTUs that obviously shifted were part of *Proteobacteria* and *Acidobacteria* (Fig. 3h), indicating that the differences in gut microbiota were mostly contributed by *Bacteroidetes* and *Firmicutes*.

### Liver-specific knockdown of ANGPTL8 regulated the functional divergence of the gut microbiome

To capture the relative abundances of the KEGG pathways related to the microbiome in the control and sh-ANGPTL8 groups, we predicted metagenome functional content based on the 16S rDNA sequences using the Tax4Fun software package. The principal component analysis (PCA) indicated that there was a significant difference in the functional profiles of the microbiomes between each group (Fig. 4a). At 'KEGG level 2', we compared the relative proportions of genes encoding

enzymes that are relevant to the KEGG functional pathways, observing that the sh-ANGPTL8 group revealed more functional pathways in replication, repair, translation, transport, catabolism and energy metabolism than the controls group (Fig. 4b and Supplemental Table 3). The results indicated that the microbiome in the sh-



microbiota between controls and sh-ANGPTL8. **a** PCA analysis of the gut microbiota between controls and sh-ANGPTL8 based on the relative abundance of KEGG pathways (KEGG L2). **b** Comparation of the relative abundance of KEGG pathways (KEGG L2) in controls and sh-ANGPTL8 based on *T* test

ANGPTL8 group probably showed higher vitality and consumed more energy during mouse ingestion, decreasing the mice's storing of energy.

### Discussion

ANGPTL8/betatrophin has shown its considerable association with diabetes and obesity as well as various metabolic biomarkers in glucose and lipid homeostasis (Wang et al. 2016; Abu-Farha et al. 2017). This hormone is reported to be highly enriched in human and mouse liver and adipose tissues (Chen et al. 2019; von Loeffelholz et al. 2017; Catalano-Iniesta et al. 2020; Akimoto et al. 2019; Mysore et al. 2017). Previous studies focused on the roles of ANGPTL8 as a hormone that directly works on the endocrine system, while hardly any evidence showed that external mediators are involved in these processes (Siddiga et al. 2017; Wang et al. 2016; Abu-Farha et al. 2017; Wang et al. 2017). To explore the effects of the relationship between the expression of ANGPTL8 and gut microbiota, we performed pyrosequencing on 16S rDNA from mouse faecal microbiota. 16S rDNA sequencing is one of the high-throughputsequencing-based methods that has been widely used in analysis of gut microbiota. By using 16S rDNA sequencing, almost all the bacterial species in the gut microbiota were able to be quantified (Sogin et al. 2006).

The results suggested that the species evenness of the gut symbionts adapted to the alteration of specific hormone levels changed during the liver-specific ANGPTL8 knockdown. Comparing the alpha diversity levels between the two faecal strains, there was no significant difference in the observed species and Chao1 indices. However, the sh-ANGPTL8 group was observed to have decreased Simpson and Shannon indices (Fig. 1). Generally, Chao1 and observed species focus on the richness of the microorganism community, while the Shannon and Simpson indices emphasise both the richness and evenness (del Valle and Astorkiza 2018; Whittaker 2019). In this study, the sh-ANGPTL8 group was shown to have a lower evenness but comparable richness as the control group. This was possibly due to the high content of Bacteroidetes in the faeces of the sh-ANGPTL8 group. Our results also revealed that there was a significant difference in the structure of the gut symbionts between the two faecal strains (Fig. 2). These results indicated that the gut microbiome might contribute to the regulation from ANGPTL8 in mice.

To further prove the role of the gut microbiome in the regulation from ANGPTL8 towards metabolism in mice, we analysed the bacterial composition and performed functional prediction of the microbiome based on KEGG. The results of bacterial composition analysis showed that there were significant differences in the gut symbiont composition between the control group and the sh-ANGPTL8 group. The main microbial phylum, Bacteroidetes, comprised approximately 70-80% of the whole microbial community; however, a lower relative F/B ratio and fewer Proteobacteria were present in the faeces of the sh-ANGPTL8 group compared with the control group (Fig. 3). Generally, gut microbiota, especially Firmicutes and Bacteroidetes, are highly associated with the regulation of host lipid, carbohydrate, and bile acid metabolism (Chen et al. 2018). In obese individuals, the increase of the relative F/B ratio generates an enhanced capacity to collect energy from food as well as a low-grade inflammation (Clemente et al. 2012), which elevates the risks of the development of NAFLD (Stols-Goncalves et al. 2019). Consistently, in patients with sustained and distinct weight loss, increased Bacteroidetes and decreased Firmicutes were identified in the gut microbiota when compared with healthy cohorts. In addition, the expansion of the population of Proteobacteria manifests a common feature of disruption of the gut microbiota composition (dysbiosis), which is associated with intestinal inflammation (Shin et al. 2015). It is noteworthy that dysbiosis has been reported as a major risk factor for the development and progression of NAFLD (Moreira et al. 2018). Proteobacteria belongs to the Gram-negative bacteria, with an outer membrane containing lipopolysaccharide (LPS) (Mukhopadhya et al. 2012). It is well known that LPS elevates the production proinflammatory cytokines, such as TNF- $\alpha$ , IL- $1\beta$  and IL-6, through activating dendritic and macrophages (Jeong et al. 2019). The increased chronic inflammation level is highly associated with metabolic syndrome and the development of T2DM (Lopez-Candales et al. 2017). In addition, the chronic inflammation induced by LPS will also increase the risk for NAFLD developing into NASH (Stols-Goncalves et al. 2019). In the present study, the reduction of the F/B ratio and the dramatically decreased abundance of Proteobacteria in the gut microbiota of the sh-ANGPTL8 group indicated that the alteration of gut microbiota composition was conducive to impede the collection of energy and decrease the levels of chronic inflammation.

According to the results of functional prediction of the microbiome based on KEGG, we found that there were significant differences between the WT and liver-specific ANGPTL8 knockdown groups in microbiome function. Functional prediction indicated that the gut microbiome in the treatment group had higher vitality and consumed more energy ingested by mice (Fig. 4). The energy consumption depends on both microbiome and host, and there is a consumption balance in between. Thus, higher energy consumption of the gut microbiome reduces energy storage. Additionally, metabolic diseases are usually accompanied by chronic inflammation. Secondary metabolites of the gut microbiome, including short-chain fatty acids (SCFAs) such as acetate, propionate and butyrate (Parada Venegas et al. 2019), have a positive effect on improving chronic inflammatory diseases and facilitating colonocyte health. Our data suggested a higher vitality of the gut microbiota in the liver-specific ANGP TL8 knockdown group, which makes it possible to provide more secondary metabolites for the host's metabolic system. Herein, the bacterial composition and functional pathways of the microbiome revealed that gut microbiota regulated by ANGPTL8 might contribute to the metabolic balance in mice.

ANGPTL8 has been reported to play important roles in carbohydrate metabolism, lipid metabolism and regulation of insulin signals and is associated with metabolic diseases, such as T1D, T2D, obesity and NAFLD (Wang et al. 2013; Abu-Farha et al. 2017; Wang et al. 2016; Lee et al. 2016). However, the potential relationship between ANGPTL8 and gut microbiota had never been demonstrated clearly. In the present study, the results illustrated that ANGP TL8 is a possible mediator of the crosstalk between the liver and the gut. There are multiple intestinal factors that act on the liver through the portal circulation, such as LPS and SCFAs, whereas bile acids are the only well-established hepatogenic molecules that are able to regulate the gut microbiota from the liver (Quesada-Vazquez et al. 2020). ANGPTL8 is a novel hepatokine that exists in the circulatory system and is regarded as a hormone that is directly associated with the endocrine system. However, our results indicated that ANGPTL8 was another possible hepatogenic protein with the capacity to modulate the gut microbiota except for bile acids. According to previous studies, hormones demonstrating the capacity to regulate the gut microbiota are almost intestinal molecules in the gut-liver axis (Mishra et al. 2016). ANGPTL8 might be additional evidence for the gut microbiota responding to the hormones secreted from remote organs in addition to sex hormones (Santos-Marcos et al. 2018; Sovijit et al. 2019; Shin et al. 2019). In conclusion, this research indicated that ANGPTL8, a kind of hepatokine, regulates the structure of the gut microbiota in mice. However, more research is needed to establish the mechanistic patterns of regulatory effects on gut microbiota by ANGPTL8.

### Supplementary information

Supplementary information accompanies this paper at https://doi.org/10. 1186/s13213-020-01599-0.

Additional file 1: Supplemental Table 1. Lists of primer sequences. Supplemental Table 2. Relative abundance of the top 10 abundant bacterial phyla in Fig. 3a and b. Supplemental Table 3. Relative abundance of KEGG pathways in Fig. 4b.

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### Authors' contributions

The authors read and approved the final manuscript.

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#### Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

#### Ethics approval and consent to participate

All animal procedures in this investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85–23, revised 1996) and was approved by the Laboratory Animal Care & Use Committee at China Pharmaceutical University (Permit number SYXK-2016–0011).

#### Consent for publication

Not applicable.

### **Competing interests**

The authors declare that they have no competing interests.

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