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BENEFICIAL MICROBES 15 (2024) 83–96

Beneficial Microbes
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REVIEW ARTICLE

The molecular mechanisms underlying gut microbiota-miRNA interaction in metabolic disorders

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Received 20 September 2023 | Accepted 21 December 2023 | Published online 29 January 2024 | Published in issue 10 February 2024

Abstract

Metabolic disorders are a major global health problem. Gut microbiota not only affect host metabolism through metabolites, inflammatory processes, and microbial-derived extracellular vesicles, but they also modulate the host microRNA, which may impact the host metabolism. Hence, the underlying mechanisms between gut microbiota-microRNA interaction can potentially be a novel alternative strategy for treating metabolic disorders. This review aims to give an update on the latest evidence and current knowledge of the underlying mechanisms of gut microbiota-miRNA interaction, focusing on metabolic homeostasis. Gut microbiota mainly communicate with host microRNA through lipopolysaccharide and secondary microbial metabolites. These signalling messengers circulate around the metabolic organs and modify gene expression through microRNA interference. Interestingly, while intestinal microRNAs play a vital role in both intestinal barrier and gut microbiota homeostasis, the presence of gut microbiota is also required for the proper functioning of intestinal microRNAs, suggesting a cooperative mechanism in intestinal health. Although the correlations between gut microbiota and microRNA have been observed in both mice and humans, a causal relationship should be confirmed. Moreover, further investigation is needed to provide more evidence of a gut microbiota-microRNA interaction to support the possibility of using that axis as a novel therapeutic target to treat metabolic disorders.

Keywords

microRNA – gut microbiota – metabolic syndrome – chronic inflammation – host-microbe communication

1 Introduction

Metabolic disorders, diseases caused by metabolic imbalance, including type 2 diabetes (T2DM), dyslipidaemia (DLP), hypertension, metabolic-associated fatty liver disease, and obesity, are currently a global health problem. The prevalence of these diseases have been increasing in the past two decades (Hirode and Wong, 2020; Kang *et al.*, 2020), even in the young adult (Al-Shehri *et al.*, 2021) and paediatric populations (Song *et al.*, 2022). Moreover, these metabolic disorders increase the risk of ischemic heart disease, which has been a worldwide leading cause of death since 2000 (Nowbar *et al.*, 2019). Therefore, the primary and secondary prevention of metabolic disorders is essential to reduce the number of ischemic heart disease cases.

Published with license by Koninklijke Brill NV | DOI: 10.1163/18762891-20230103
 | ISSN: 1876-2883 (print) 1876-2891 (online)

Gut microbiota, the microorganisms colonising the gastrointestinal tract, have been reported to be associated with metabolic disorders. Loss of gastrointestinal microbial equilibrium, also known as gut dysbiosis, has been found in people with T2DM (Wu *et al.*, 2020) and metabolic syndrome (MetS) (León Aguilera *et al.*, 2022), which is a group of risk factors for T2DM and cardiovascular disease (CVD). As a result, the potential role of gut microbiota as both therapeutics and targets for metabolic disorder prevention has been proposed (León Aguilera *et al.*, 2022; Mutalub *et al.*, 2022). Lipopolysaccharide (LPS), a gram-negative microbial component, and microbial metabolites, e.g. short-chain fatty acids (SCFA), indole-3-propionic acid (IPA), and secondary bile acids, are the primary signalling messengers in the communication between gut microbiota and the host (Salazar *et al.*, 2020; Sikalidis and Maykish, 2020). Apart from these mechanisms, epigenetic modulations by gut microbiota that influence the host's metabolism have received a lot of attention in recent years.

MicroRNA (miRNA), which are short, non-coding, single-stranded RNA, act as epigenetic modulators and various miRNAs have been extensively investigated for their regulatory role in lipid and glucose metabolism in the past decade (Du *et al.*, 2021a). After being synthesised, the interaction between miRNA and a messenger RNA (mRNA) target leads to either mRNA translational repression or degradation depending on the miRNA-mRNA complementarity. Each miRNA may have one or more mRNA targets. Various miRNAs and their metabolism-related target genes have been validated, suggesting that miRNA may be a promising therapeutic option for metabolic disorders (Desgagné *et al.*, 2017; Du *et al.*, 2021a).

The association between miRNA and gut microbiota has been widely studied recently. Gut microbiota can modulate the host miRNA and be regulated by either host miRNA or diet-derived miRNA (Bi *et al.*, 2020). Although the conclusive mechanism of miRNA and gut microbiota interaction has not yet been clarified, several pathways have been proposed (Behrouzi *et al.*, 2020; Wu *et al.*, 2021). These underlying mechanisms can potentially be a novel or alternative strategy for preventing and treating metabolic disorders. Therefore, this review aims to update the latest evidence for gut microbiota-miRNA interaction in metabolic disorders and the current knowledge on the underlying mechanisms focusing on metabolic homeostasis.

2 Overview of miRNA

The biogenesis of miRNA

The first miRNA was identified in 1993 (Lee *et al.*, 1993), and it was reported to be 22 nucleotides long on average. A primary miRNA (pri-miRNA) is generated with a 5′ cap and a 3′ poly (A) tail by either RNA polymerase II or RNA polymerase III in the nucleus. The structure of the hairpin loop on pri-miRNA is recognised and cleaved by DiGeorge Syndrome Critical Region 8 (DGCR8) and Drosha, a ribonuclease (RNase) III family protein, respectively, forming a precursor miRNA (pre-miRNA). The pre-miRNA is transported to the cytoplasm by exportin-5 and the Ras-related nuclear protein guanosine triphosphate (Matsuyama and Suzuki, 2019; O'Brien *et al.*, 2018).

In the cytoplasm, dicer, an RNase III family member, together with the trans-activation response RNA binding protein removes the terminal loop of the pre-miRNA hairpin, generating a mature miRNA duplex. The mature miRNA strand initiated from the 5′ end of hairpin loop is labelled with the suffix –5p, while the strand with –3p indicates the duplex of the –5p strand. Although both mature strands are loaded into the Argonaute (Ago) proteins afterward, only the guide strand is preserved. The passenger strand (miRNA*) is removed from the Ago proteins and degraded (Matsuyama and Suzuki, 2019; O'Brien *et al.*, 2018). The guide strand forms the RNA-induced silencing complex (RISC) with Ago proteins. RISC interacts with the mRNA target through the seed sequence, the 2-8 nucleotides at the 5′ end of miRNA, and the supplementary sequence, the 13-16 nucleotides at the 5′ end (Marzec, 2020). While a partial complementarity between RISC and mRNA target suppresses mRNA translation, the target mRNA is degraded upon perfect complementarity with RISC (Matsuyama and Suzuki, 2019; O'Brien *et al.*, 2018).

miRNA and host communication

In 2008, Lawrie *et al.* first reported the presence of miRNA in serum and plasma samples (Lawrie *et al.*, 2008). The levels of circulating miRNAs in humans with cancer showed no difference from the cancer xenograft-bearing mice and remained constant after exposure to plasma RNase (Mitchell *et al.*, 2008), food intake, and sampling at different times (Kupec *et al.*, 2022; Sabbaghian *et al.*, 2022). These findings brought attention to the idea that miRNA may not only be responsible for an autocrine signalling, but also may be a mediator of cell-to-cell communication (Mori *et al.*, 2019).

Apart from the communication between human cells, miRNAs present in plants and animal-sourced foods can be absorbed into the circulatory system and interfere with mammalian genes (Baier *et al.*, 2014; Zhang *et al.*, 2012). In addition, food-derived exosomes containing miRNAs can be taken up by gut microbiota and interact with bacterial mRNAs, supporting the cross-domain communicating capacity of miRNAs (Teng *et al.*, 2018). Hence, several review articles in the last few years have proposed a new role for miRNA in gut microbiota-host communication (Bi *et al.*, 2020; Du *et al.*, 2021b; Li *et al.*, 2020c). This communication between the intestinal microbiome and host cells may explain why and how the alterations to microbial composition influence the host's health.

3 Current evidence of molecular mechanisms underlying gut microbiota-miRNA interaction in host metabolic homeostasis

The association between gut microbiota and host miRNA was first published in 2011. Dalmasso *et al.* (2011) observed a difference in miRNA expression between germ-free mice and colonised mice. After this discovery, the trend of global research on gut microbiota-miRNA interaction substantially increased from 1 to 24% of this study field over the span of 10 years (Yan *et al.*, 2022). Briefly, the expression of hepatic miRNAs in animal studies was found to be significantly changed after introducing a HFD (Blasco-Baque *et al.*, 2017; Dong *et al.*, 2021; Jia *et al.*, 2018). The altered patterns of these miRNAs were consistent with the shifting of gut microbiota composition. Interestingly, many of the predicted target genes of these miRNA were involved in metabolic pathways, confirming the role of miRNAs in modulating metabolism. In line with the animal studies, correlations between gut microbiota and miRNA were reported in patients with T2DM, obesity, and MetS (Assmann *et al.*, 2020; Li *et al.*, 2020b; Wortelboer *et al.*, 2022). Although correlations between gut microbiota and miRNA were observed in MetS, the causal relationship still needs to be confirmed to support the possibility of using gut microbiota-miRNA interaction as a novel therapeutic target to treat metabolic disorders. Hence, understanding the underlying mechanisms of this interconnection is necessary.

Immunity and inflammation

McKenna *et al.* (2010) reported the presence of an association between miRNA and the intestinal barrier in 2010. Loose and fatty stools were observed in mice with genetic ablation of *Dicer1*, a gene-encoded miRNA processing protein. Correspondingly, both intestinal permeability and inflammatory cells were more prominent in *Dicer1*-deficient mice compared to non-mutant group. Although the importance of miRNA processing for intestinal homeostasis was demonstrated in this study, the expression of intestinal miR-21 was inconsistent with inflammatory bowel disease (IBD) patients (Yan *et al.*, 2020). Higher expression of intestinal miR-21 was found in both dextran sodium sulphate (DSS) colitis model mice and patients with IBD (Johnston *et al.*, 2018; Yan *et al.*, 2020), while a downregulated miR-21 was observed in *Dicer1*-deficient mice (McKenna *et al.*, 2010). The benefits of a deficiency of miR-21 were confirmed in DSS-induced colitis model mice lacking miR-21, showing less bloody stool and lower inflammation and cell damage compared to the wild-type group (Johnston *et al.*, 2018). Since the *Dicer1* gene has a crucial role in non-specific miRNA maturation (Foulkes *et al.*, 2014), there are various factors affecting intestinal homeostasis. Interestingly, the benefits of a deficiency of miR-21 in DSS-induced colitis model mice decreased after administration of antibiotics and there was a difference in gut microbiota composition observed between miR-21-knockout mice and the control group, implying a role of gut microbiota in the function of miR-21 (Johnston *et al.*, 2018). This finding supported the previous study that showed that expression of different miRNAs depended on the presence of gut microbiota (Singh *et al.*, 2012). Collectively, these results indicated that intestinal miRNA expression and gut microbiota cooperate in the regulation of intestinal inflammation.

In a comparable result to the study by Johnston *et al.* (2018), miR-21-deficient mice also showed an anti-inflammatory effect on intestinal inflammation in the bile duct ligation (BDL) surgery model (Santos *et al.*, 2020). This model has been recognised for its complications, including elevated plasma LPS, liver injury, gut dysbiosis, and metabolic disturbance (Zhou *et al.*, 2023). Although the mRNA expression of intestinal tight-junction proteins and junctional adhesion molecules were significantly decreased after BDL, in miR-21-knockout mice, the mRNA levels were maintained after BDL. In addition, the lack of miR-21 alleviated the shift in gut microbiota composition after BDL-induced liver injury and facilitated the growth of probiotic *Lactobacillus*. Lower serum endotoxin levels were reported in miR-21-deficient mice, corresponding with a stronger intestinal barrier and gut micro-

biota homeostasis. The severity of hepatic injury and fibrosis was also reduced in miR-21-knockout mice (Santos *et al.*, 2020). Likewise, the improvement of metabolic disturbance after providing an antagonist of miR-21 was confirmed in streptozotocin-induced T2DM rat (Wang *et al.*, 2020). The role of miR-21 on lipid metabolism and chronic liver diseases, e.g. non-alcoholic fatty liver disease (NAFLD) or liver fibrosis, has been well-documented (Zhang *et al.*, 2020). Therefore, these findings suggested that miR-21 deficiency has a hepatic protective effect through modulating gut microbiota, regulating the tight junctions of intestinal epithelial cells (IECs), and reducing endotoxemia (Santos *et al.*, 2020). Nonetheless, the crosstalk between gut microbiota and miR-21 on lipid metabolism should be more explored.

Similar to the previous study (Singh *et al.*, 2012), the expression of miR-21-5p in germ-free (GF) mice was markedly different from conventional mice. LPS enhanced the expression of miR-21-5p in human IEC lines and the LPS-induced miR-21-5p was suppressed with anti-TLR4 antibody treatment (Nakata *et al.*, 2017). This outcome supported the study of Frederick *et al.*, suggesting that LPS stimulates miR-21 expression through TLR4/MyD88/NF-κB (Sheedy *et al.*, 2010). The mechanism of miR-21-5p in increasing IEC permeability was further investigated. It was revealed that miR-21-5p targets PTEN, leading to the activation of the protein kinase B (Akt) and c-Jun N-terminal kinase (JNK) signalling pathway, which resulted in the augmentation of ADP ribosylation factor 4 (ARF4), a member of the family of small GTPases involved in IEC homeostasis. IEC permeability was then found to be increased. In contrast, ARF4 inhibition promoted the expression of tight junction proteins (Nakata *et al.*, 2017). In line with the above evidences, downregulated PTEN with overexpression of miR-21 was observed in the intestinal tissue of Crohn's disease patients (Wang *et al.*, 2022), which has been reported to be associated with MetS (Dragasevic *et al.*, 2020). Moreover, in a mouse model of colitis, the decreased ratio of regulatory T cells and T helper-1 cells, which plays a vital role in obesity with insulin resistance, was reversed by treatment with antagomiR-21, a synthesised antagonist of miR-21 (Liu *et al.*, 2021; Wang *et al.*, 2022). As a result, chronic inflammation in the intestine and gut dysbiosis-related LPS may aggravate the progression of metabolic disorders through miR-21 resulting in impaired permeability of IECs and induction of a systemic inflammatory response.

Apart from the importance of miRNA in impairing the intestinal barrier, miRNA also modulates the inflammation caused by LPS from gut dysbiosis (Anzola *et al.*, 2018). The benefits of miR-146a on anti-inflammation and metabolic disorders have been reported in several studies (Li *et al.*, 2020a; Runtsch *et al.*, 2019). The level of IEC miR-146a was substantially elevated with the highest response to LPS stimulation. Upon the binding of LPS with TLR4, MyD88, a primary downstream adapter of TLR, is triggered and subsequently activates the NF-κB and Akt pathways. Since the inhibition in each step of the TLR4/MyD88/NF-κB-Akt pathway resulted in downregulation of both pre- and mature miR-146a, the underlying mechanism of miR-146a overexpression induced by LPS is likely to be this pathway. In addition, the overexpression of miR-146a eventually repressed monocyte chemoattractant protein-1 (MCP-1) secretion, which is a chemokine that promotes inflammatory cell infiltration. These outcomes confirm that LPS stimulates miR-146a expression through the TLR4/MyD88/NF-κB-Akt pathway, and the miR-146a subsequently regulates inflammatory processes. Interestingly, upregulated expression of miR-146a was found in acute colitis-induced mice, but not in a chronic inflammation-induced model. These results imply that the degree of intestinal barrier damage, which is more severe in the acute model, also plays a role in the appropriate response of miRNA suppression of inflammation. On the other hand, the regulatory function of miR-146a may be impaired in the setting of chronic inflammation (Anzola *et al.*, 2018). The dysfunction of anti-inflammatory miRNA may be one of the causes of chronic inflammatory diseases and, thus, has the potential to be a novel approach to disease prevention and therapy. Besides, since LPS stimulated the anti-inflammatory miR-146a expression, the importance of gut homeostasis should be highlighted.

The link between gut microbiota composition, impaired intestinal barrier, circulating miRNA, and hepatic inflammation in the development of NAFLD was observed in Sprague Dawley rats. The bacterial phyla *Tenericutes* and *Cyanobacteria* were observed to be more abundant in the control group, while *Bacteroidetes* and *Proteobacteria*, which are both gram-negative bacteria containing LPS, were more prominent in the NAFLD group. Accordingly, genes involved with tight junction proteins, including occludin (*Ocln*) and F11 receptor (*F11r*), were markedly downregulated in the NAFLD-induced rats. Furthermore, the expression of hepatic TLR4 and MyD88 were significantly increased in the NAFLD group, which was consistent with the increased expression of tumour necrosis factor (TNF)-α gene and hepatic concentration. These outcomes demonstrate a

potential role for gut dysbiosis-derived LPS in hepatic inflammation, which was also correlated with NAFLD progression. Although relationships between NAFLD and circulating miR-122 have been observed, there was no mention of a link to inflammation in this study (Jia *et al.*, 2018). Comparable with the results from the NAFLD rat model, higher serum and plasma miR-122 were found in human subjects with MetS and T2DM (Willeit *et al.*, 2017). This evidence proposed a promising role of miR-122 as a predictor of MetS and T2DM development. Willeit *et al.* (2017) also confirmed the effect of miR-122 in an inhibited miR-122 mice model using antagomiR-122. After treatment with antagomiR-122, the expression of genes involved in lipid biosynthesis, including ATP citrate lyase (*Acly*) and sterol regulatory element-binding protein 1 (*Srebp1*), and triglyceride transportation, including microsomal triglyceride transfer protein (*Mttp*), were all declined. As expected, a substantially reduced total cholesterol level was observed in mice that had been treated with antagomiR-122 (Willeit *et al.*, 2017). Taken together, the results suggest that there may be a connection between gut dysbiosis-derived systemic LPS, IEC impairment, and miR-122 upregulation that ultimately promotes lipid metabolism disturbance and NAFLD progression. The potential interplay between host miRNAs and gut microbiota on metabolic disorders via inflammatory response and gut microbiota modulation is summarised in Figure 1.

Blasco-Baque *et al.* also reported evidence of LPS specifically stimulating hepatic miRNA expression in primary hepatocytes of Cd14 knockout mice. LPS was found to induce miR-181a, miR-666, and miR-21 expression. However, only miR-666 and miR-21 were statistically negatively correlated with hepatic triglyceride concentration. In this model, while *Firmicutes* were positively associated with liver triglyceride level, *Bacteroides acidifaciens* showed the strongest negative relationship with hepatic triglyceride concentration. In accordance with these observed correlations, both miR-666 and miR-21 had an inverse association with *Firmicutes* and a positive relationship with *B. acidifaciens*. These findings not only suggested a link between gut microbiota, miRNA, and hepatic triglycerides, but also emphasised the potential impact of LPS on gut dysbiosis-hepatic miRNA communication (Blasco-Baque *et al.*, 2017). In addition, miR-21 in a macrophage-like cell line was also found to be elevated with LPS incubation in a dose- and time-dependent manner. The upregulated expression of miR-21 in macrophages inhibited both fat accumulation and oxidised-LDL (ox-LDL) uptake in the macrophages. As a result of the overexpression of miR-21 induced by LPS, foam cell formation, a hallmark of atherosclerosis progression, was inhibited. Furthermore, the augmentation of miR-21 suppressed the TLR4/NF-κB signalling pathway in macrophage, leading to a reduction in the production of the pro-inflammatory cytokine interleukin (IL)-6, and an increase in production of the anti-inflammatory cytokine IL-10. The beneficial effects of miR-21 on preventing inflammation were also supported by the results of anti-miR-21 inhibitor transfection in another experiment (Feng *et al.*, 2014). Hence, miR-21 may play a role in atherosclerosis progression, which is a primary consequence of metabolic disorders related to chronic inflammation (Hasheminasabgorji and Jha, 2021). Nonetheless, the positive effects of miR-21 were contrary to the aforementioned evidences (Nakata *et al.*, 2017; Santos *et al.*, 2020; Wang *et al.*, 2020; Wang *et al.*, 2022). The functions of miRNA in each type of cell may be various. Prior to clinical trials, a further investigation for a more in-depth mechanism is required.

Microbial metabolites

The alteration of gut microbiota is not only related to inflammation, but it also influences microbial metabolite shifting, resulting in metabolic disturbance. The abundance of SCFA producers, *Prevotella* and *Bifidobacterium*, were reported to be higher in miR-21-deficient mice (Johnston *et al.*, 2018). Since the depletion of these genera was also detected in T2DM patients (Cunningham *et al.*, 2021), it is possible that miR-21 may influence host metabolism via gut microbiota modulation altering microbial metabolites in these patients. Nevertheless, the causal relationship between miR-21 and these beneficial microbes should be confirmed *in vitro* study. Interestingly, synthetic human miR-21 inhibited the growth of probiotic *Lactobacillus spp*. in an *in vitro* study, and this probiotic was also found to be prominent in miR-21-knockout mice (Santos *et al.*, 2020). This probiotic has been extensively reported on for its positive effect in liver diseases, including NAFLD and liver fibrosis (Ritze *et al.*, 2014; Santos *et al.*, 2020). Unsurprisingly, an improvement in hepatic inflammation was observed in miR-21-deficient mice (Santos *et al.*, 2020). D-lactate, the main product of *Lactobacillus spp*. metabolised by D-lactate dehydrogenase (D-LDH), can either be a fuel for cellular metabolism or a substrate for other SCFAs production (Ewaschuk *et al.*, 2005; Santos *et al.*, 2020). Although serum D-lactate was not detected, the hepatic D-LDH mRNA was significantly upregulated in mice lacking miR-21. Santos *et al.*, thereby, suggested that the suppression of *Lactobacillus spp*. growth and subse-

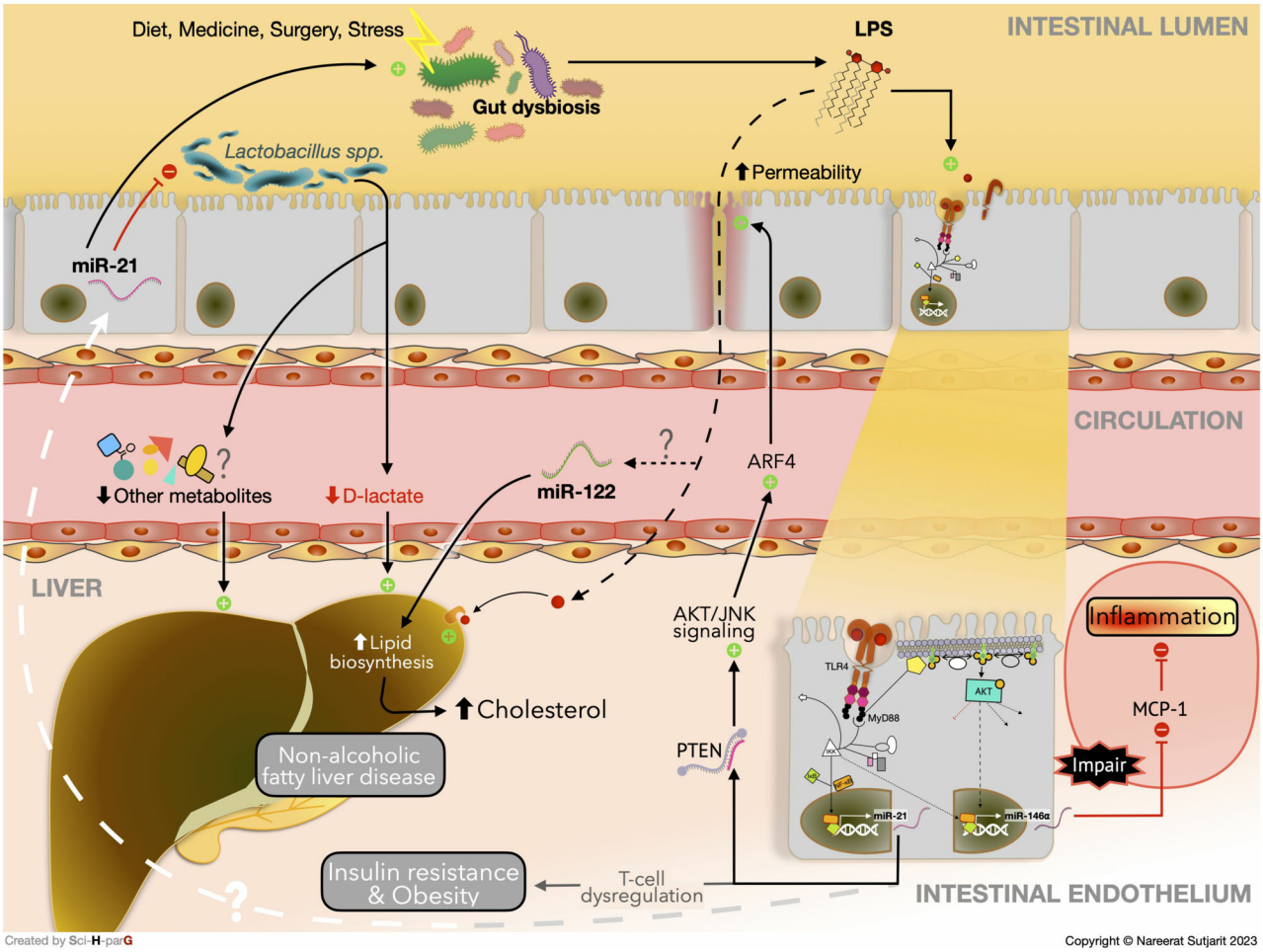

FIGURE 1 The potential interplay between host miRNAs and gut microbiota on metabolic disorders via inflammatory response and gut microbiota modulation.

quent reduction of D-lactate caused by miR-21 aggravate liver injury (Santos *et al.*, 2020). Although, the potential benefits of D-lactate on liver fibrosis was mentioned in this study, several recent studies observed the positive correlations between D-lactate on chronic liver diseases and impaired intestinal barrier (Peng *et al.*, 2018; Zhang *et al.*, 2023). The favourable effects of miR-21 deficiency promoting *Lactobacillus spp*. growth may due to other microbial metabolites, e.g. acetate, propionate, and butyrate (Markowiak-Kopeć and Śliżewska, 2020) (Figure 1).

Butyrate, one of the most well-known microbial metabolite products, is absorbed by enterocytes and subsequently binds to GPR43 on multiple target organs, modulating host metabolism (Coppola *et al.*, 2021). Although Pant *et al.* reported a beneficial role of butyrate in liver cancer through sirtuin 1 (SIRT1) inhibition, which was found to be mediated by upregulation of miR-22 expression (Pant *et al.*, 2017), another study showed that the overexpression of miR-22 promoted hepatic steatosis via inhibition of fibroblast growth factor 21 (FGF21) (Hu *et al.*, 2020). Furthermore, butyrate-mediated suppression of histone deacetylase 3 (HDAC3) resulting in increased expression of FGF21, a peptide hormone regulating glucose and lipid metabolism, has also been reported (Li *et al.*, 2012). These studies denote the action of butyrate on different epigenetic modulations under distinct circumstances. Interestingly, a recent study reported evidence of butyrate interfering with a post-transcriptional regulator of miRNA expression (Das *et al.*, 2022). After the adenylate and uridylate (AU)-rich element binding factor 1 (AUF1) interacts with AU-rich elements (AREs) on the 3′-UTR of target mRNA, the mRNA becomes unstable, resulting in diminished mRNA translation and even mRNA degradation. *Dicer1* mRNA segments can be targets for AUF1. Since *Dicer1* mRNA encodes the dicer enzyme, which is critical for miRNA maturation, lower overall miRNA levels were reported after AUF1 overexpression (Abdelmohsen *et al.*, 2012). Das *et al.* observed that butyrate has a role in AUF1 upregulation, leading to *Dicer1* downregulation and ultimately repressing hepatic miR-122 expression.

Lipid biogenesis-related genes, such as HMG CoA reductase, 7-dehydrocholesterol reductase, and HMG CoA synthase 1, have been reported to be targets of miR-122; hence, lower cellular and serum cholesterol levels were observed in Huh7 cells and in mice fed with a HFD, respectively (Das *et al.*, 2022).

IPA, a tryptophan-derived microbial metabolite, has been well-recognised as an inflammation suppressor, intestinal barrier protector, and host metabolic modulator (Sehgal *et al.*, 2022). A negative correlation between IPA level and CVD risk has been reported, and it was also found that the gut microbiota producing IPA, especially *Clostridium* and *Peptostreptococcus*, were reduced in patients with coronary artery disease compared to healthy individuals (Xue *et al.*, 2022). The depletion of these bacterial genera also reduced the serum IPA level by more than 50% in mice fed a Western diet. Treatment with IPA attenuated fat accumulation in both human and mouse macrophages in a dose-dependent manner by promoting cholesterol efflux to ApoA1. ATP-binding cassette transporter A1 (ABCA1), the primary lipid transporter in macrophages, was observed to be the main channel of cholesterol efflux induced by IPA. Interestingly, the expression of transcriptional factor SPI1 was reduced after IPA treatment. Under normal circumstances, this transcriptional factor upregulates miR-142-5p expression, which interacts with ABCA1. Taken together, it is thought that IPA treatment enhanced macrophage cholesterol efflux by inhibiting SPI1, downregulating miR-142-5p, and ultimately promoting ABCA1 expression. The alleviation of atherosclerotic plaques was confirmed in an animal study, corresponding with the above mechanism (Xue *et al.*, 2022). Moreover, another tryptophan-derived metabolite, indole, was found to specifically impact miR-181 expression in WAT without LPS involvement (Virtue *et al.*, 2019). It was also found that miR-181-knockout in macrophages and hepatocytes had no impact on weight gain and insulin resistance. Likewise, after receiving a HFD, increased expression of miR-181 was only detected in adipocytes of obese mice, thereby implying that miR-181 specifically acts on adipocytes in obesity and insulin resistance. Additionally, downregulation of miR-181 in WAT was revealed only in the presence of *Escherichia coli* strains carrying the enzyme tryptophanase (TnaA), which catalyses the conversion of tryptophan into indole. Correspondingly, mice fed with a HFD and colonised with TnaA-knockout *E. coli* strains had aggravated weight gain and impaired glucose tolerance compared with the control group (Virtue *et al.*, 2019). These results denote a link between gut microbiota-derived metabolites, host miRNA, and metabolic homeostasis.

Not all microbial metabolites yield benefits for host metabolism. Trimethylamine N-oxide (TMAO), a primary metabolite derived from animal products, has been recognised as a pro-CVD factor (Canyelles *et al.*, 2023). TMAO was found to promote the expression of miR-17-92 cluster in various cell lines (Díez-Ricote *et al.*, 2022). Serpin Family E Member 1 (*SERPINE1*), one of the mRNA targets of miR-17-92 cluster, has been reported to have a positive correlation with atherogenesis (Kubota *et al.*, 2021). Although the expression of *SERPINE1* and IL-12A were upregulated with TMAO treatment, in the same way as miR-17-92 cluster, there was no further investigation to prove the direct influence of miR-17-92 cluster on *SERPINE1* and IL-12A expression (Díez-Ricote *et al.*, 2022). Since animal products are a primary source of high biological value proteins, which are necessary for human development (Giromini and Givens, 2022), further study should be done to confirm the role of TMAO on miRNA, expanding the prevention perspective on TMAO-related metabolic diseases.

Apart from the above well-known microbial metabolites, compounds produced by fermentation of phytochemicals by gut microbiota have also been shown to be involved in the regulation of metabolic homeostasis and inflammation. Urolithin A, a bioactive compound from ellagitannins and ellagic acid, significantly decreased miR-10, miR-99b, miR-146a, and miR-155 expression after LPS stimulation and inhibited NF-kB activation (Abdelazeem *et al.*, 2021). The repression of IκBα phosphorylation, mitogen activated protein kinase (MAPK), and Akt/mechanistic target of rapamycin (mTOR) signalling pathways were found to be the underlying mechanisms of urolithin alleviating inflammation (Abdelazeem *et al.*, 2021). Since miR-146a is strongly associated with LPS-induced gut inflammation through the TLR4/MyD88/NF-κB-Akt pathway as mentioned above (Anzola *et al.*, 2018), the anti-inflammatory role of urolithin A via miRNA regulation was indicated as it suppressed miR-146a expression (Abdelazeem *et al.*, 2021). In addition to urolithin A, another bioactive compound derived from anthocyanins and metabolised by gut microbiota, protocatechuic acid, has an impact on metabolism and atherogenesis by enhancing macrophage cholesterol efflux (Wang *et al.*, 2012) similar to the study with IPA mentioned above from Xue *et al.* (2022). However, in the case of protocatechuic acid, the active miRNA was distinct. Protocatechuic acid reduced miR-10b expression and directly targeted ABCA1 and ATP Binding Cas-

sette Subfamily G Member 1. The upregulation of these genes enhanced cholesterol efflux from macrophages and thus, alleviated foam cell formation (Wang *et al.*, 2012).

Microbial-derived extracellular vesicles

Similar to human extracellular vesicles (EVs), microbial-derived extracellular vesicles (MEVs) contain genetic information, e.g. miRNA or mRNA, and carry their genetic cargo to the target cell and subsequently initiate communication by either ligand-receptor interaction, specific surface antigen-receptor interaction, or membrane fusion (Martellucci *et al.*, 2020; Nik Mohamed Kamal and Shahidan, 2020). Although the research into the disruption of metabolic homeostasis induced by miRNA in gut MEVs is scarce, there are a few reports of the effects of oral microbiota-derived EVs on host health (Choi *et al.*, 2017; Han *et al.*, 2019). Like IBD, chronic periodontitis can interfere with host health via oral microbial dysbiosis, barrier impairment, and chronic systemic inflammation (Byrd and Gulati, 2021). The release of miRNA-size small RNA (msRNA) from outer-membrane vesicles (OMVs) derived from immuno-interfering periodontal pathogens, including *Aggregatibacter actinomycetemcomitans* (*Aa*), *Porphyromonas gingivalis*, and *Treponema denticola*, has been observed. As expected, several predicted gene targets of these msRNAs are involved with the immune system. Moreover, the expression of IL-5, IL-13, and IL-15 was substantially decreased after synthetic msRNA transfection of T-cells (Choi *et al.*, 2017). Research by Han *et al.* (2019) also found that the presence of OMVs derived from *Aa* significantly increased TNF-α secretion from macrophages, while the presence of lysed OMVs showed no significant change. This study suggests the importance of OMVs on bacterial genetic protection and the presence of an inter-kingdom communication channel by direct uptake to the macrophage. After entering the macrophage, the msRNA of *Aa* triggered TLR8 and activated the NF-ϰB signalling pathway and the expression of TNF-α protein was subsequently elevated. In addition, the msRNA was able to interact with host Ago2 and form RISC. Therefore, the msRNA from periodontal pathogens may be able to impact host gene expression through interaction with Ago2 and RISC formation. OMVs from *Aa* were also found to be able to cross the blood-brain barrier and were able to eventually promote TNF-α in the brain (Han *et al.*, 2019).

Even though a large number of studies have been carried out in recent years on the impacts of MEVs on host metabolism, the majority of research has focused on the presence of MEVs, LPS, and microbial DNA (Castaño *et al.*, 2023). Consistent with the above mechanisms, MEVs derived from beneficial gut microbiota, such as *Akkermansia muciniphila*, *Lactobacillus spp.*, and *Propionibacterium freudenreichii*, ameliorated intestinal inflammation (Choi *et al.*, 2020; Rodovalho *et al.*, 2020), regulated IEC tight junctions (Chelakkot *et al.*, 2018), and, ultimately, deferred the development of obesity in mice fed with a HFD (Ashrafian *et al.*, 2021). Interestingly, although the abundance of *Pseudomonas panacis* and *Pseudomonas cedrina* were similar between mice fed a HFD and the regular diet group, the MEVs derived from these two pathogens were significantly different. MEVs released from *P. panacis*, which were more prominent in HFD-fed mice, contained more LPS than MEVs from *P. cedrina*. Furthermore, the *P. panacis*-derived MEVs promoted insulin resistance by inhibiting Akt signalling pathway in adipocytes and suppressed GLUT-4 translocation in skeletal muscle (Choi *et al.*, 2015). This result corresponded with the study by Nah *et al.*, as there was a correlation between faecal, blood, and urine MEVs in patients with T2DM (Nah *et al.*, 2019). Collectively, these findings support the importance of MEVs derived from gut microbiota in host metabolic homeostasis.

4 Conclusions

According to the current evidence, gut microbiota not only influence the host metabolism through the immune system, microbial metabolite, gut hormone, or nervous system (Cook and Mansuy-Aubert, 2022), they can also modulate host metabolic homeostasis via post-transcriptional regulation of a gene in target organs. The signalling messengers of gut microbiota, including LPS, MEVs, and gut microbiota-derived metabolites, play a key role in this epigenetic control (Figure 2). The discovery of gut microbiota specifically interfering with host miRNA in each metabolic organ may provide a novel solution for managing metabolic disorders. Nevertheless, further investigation is needed to provide more evidence for this novel therapeutic strategy.

Authors' contribution

Conceptualisation: PP and NS; writing-original draft preparation: PP; writing-review and editing: PP, NS, and JS. All authors have read and agreed to the published version of the manuscript.

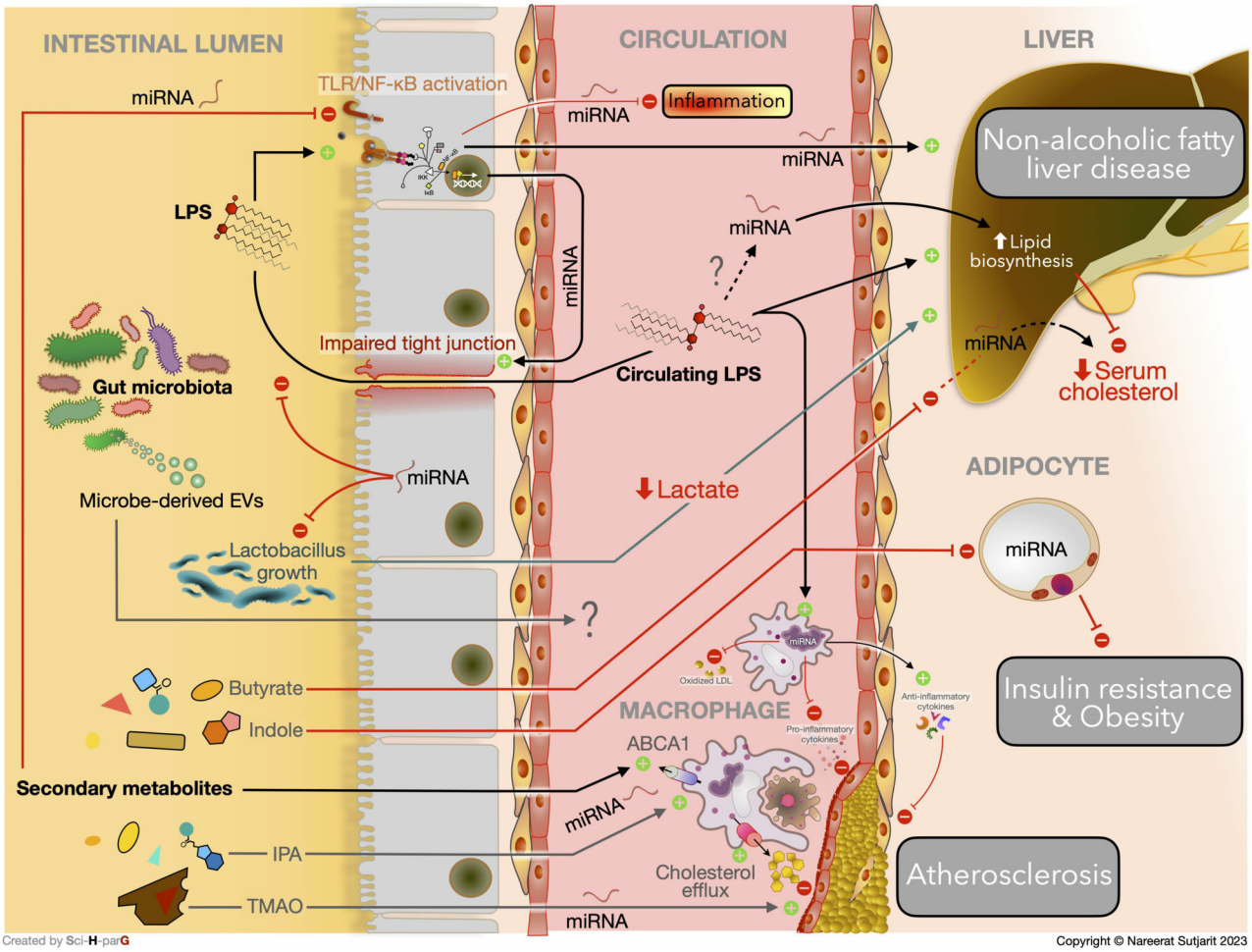

FIGURE 2 Current evidence of molecular mechanisms underlying gut microbiota-miRNA interaction in host metabolic homeostasis.

Conflict of interest

The authors declare no conflict of interest.

Funding

This research received no external funding.

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