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REVIEW ARTICLE

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Gut microbiota crosstalk mechanisms are key in pulmonary hypertension: The involvement of melatonin is instrumental too

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Abstract

The microbiota refers to a plethora of microorganisms with a gene pool of approximately three million, which inhabits the human gastrointestinal tract or gut. The latter, not only promotes the transport of nutrients, ions, and fluids from the lumen to the internal environment but is linked with the development of diseases including coronary artery disease, heart failure, and lung diseases. The exact mechanism of how the microbiota achieves crosstalk between itself and distant organs/tissues is not clear, but factors released to other organs may play a role, like inflammatory and genetic factors, and now we highlight melatonin as a novel mediator of the gut-lung crosstalk. Melatonin is present in high concentrations in the gut and the lung and has recently been linked to the pathogenesis of pulmonary hypertension (PH). In this comprehensive review of the literature, we suggest that melatonin is an important link between the gut microbiota and the development of PH (where suppressed melatonin-crosstalk between the gut and lungs could promote the development of PH). More studies are needed to investigate the link between the gut microbiota, melatonin and PH. Studies could also investigate whether microbiota genes play a role in the epigenetic aspects of PH. This is relevant because, for example, dysbiosis (caused by epigenetic factors) could reduce melatonin signaling between the gut and lungs, reduce subcellular melatonin concentrations in the gut/lungs, or reduce melatonin serum levels secondary to epigenetic factors. This area of research is largely unexplored and further studies are warranted.

KEYWORDS
gut microbiota, inflammation, melatonin, pulmonary hypertension, signaling pathways

INTRODUCTION

The microbiota comprises myriad microorganisms including protozoans, archaea, fungi, and bacteria[1] that inhabit the human gastrointestinal tract or gut.[2] Gut microbiota contains microorganisms with a gene pool of approximately three million genes.[3,4] Moreover, gut microbiota contains digestive enzymes that help the body metabolize animal and plant carbohydrates,[5] and it can produce short-chain fatty acids that support beta-oxidation.[6] These features of the microbiota allow it to code for thousands of physiological factors that are kept in homeostatic balance through microbiome-host interactions[7] whereas an imbalance can also contribute to some diseases.[8] Gut microbiota can release these factors (which could be inflammatory, genetic, or metabolic) into the circulation where they can reach and impact[3,4] other parts of the body[9] describe in this paper as crosstalk.[10] This is observed for example, when short-chain fatty acids (butyrate, propionate, and acetate) are produced by bacterial fermentation of indigestible polysaccharides and triggers the release of the satietogenic hormone glucagon-like peptide-1 from enteroendocrine L-cells.[11] These hormones travel through the body via blood circulation to the hypothalamus where they can regulate ingestive behavior.[11] On the other hand, propionate, butyrate, and acetate modulate the metabolism and function of these tissues.[12] The gut microbiota is instrumental in the homeostasis of nutrient metabolism and energy production, by supporting oxidative metabolic pathways via this crosstalk action.[13] Needless to say, a homeostatic balance exists between "good and bad" organisms, to promote good health.[14] Herein lies two important concepts, eubiosis,[15] which occurs when good organisms prevail over the bad, and dysbiosis, which is the disbalance in the microbiota that predisposes to sickness.[15] Dysbiosis may occur due to disturbances in the microbiome caused by various lifestyle and environmental factors.[16–19] Also with regard to dysbiosis,[20,21] the literature supports the notion that it can contribute to the development and progression of heart and lung diseases.[19,22]

THE ROLE OF GUT MICROBIOTA IN MAINTAINING IN HEALTH

The gut microbiota plays a major role in human health through its ability to influence immune and metabolic health, neuro- and cardiovascular-related functions.[23] Organisms that form part of the gut microbiota[24] are too many to discuss in this paper, but we present an abridged list in Table 1. Some of the most well-known species include *Clostridium spp*., *Lactobacillus reuteri*, *Enterococcus faecium, Bifidobacterium bifidum, and Escherichia coli*.[25] These organisms are believed to enter the human host at birth, via the amniotic and maternal fluids.[26] A study by Putignani et al.[27] demonstrated that the normal structure, composition and thus functioning of the gut microbiota promotes overall health. This suggests the importance of maintaining a balance in microbiota composition to ensure overall good health. *Clostridium*

TABLE 1 A brief overview of phyla, classes, genus, and species of microorganisms that form part of the gut microbiota.

Phylum	Class	Genus	Species
Firmicutes	Bacilli	Lactobacillus	*Lactobacillus reuteri*
		Staphylococcus	*Staphylococcus leei*
		Enterococcus	*Enterococci faecium*
	Clostridia	Clostridium	*Clostridium spp.*
		Ruminicoccus	*Ruminicoccus faecis*
Bacteroidetes	Bacteroidia	Bacteroides	*Bacteroides vulgatis*
		Prevotella	*Prevotella* spp.
Actinobacteria	Actinobacteria	Bifidobacterium	*Bifidobacterium bifidum*
			Bifidobacterium longum
Proteobacteria	Delta proteobacteria	Desulfovibrio	*Desulfovibrio intestinalis*
	Gamma proteobacteria	Escherichia	*Escherichia coli*
Fusobacteria	Fusobacteriia	Fusobacterium	*Fusobacterium nucleatum*
Verrucomicrobia	Verrucomicrobiae	Akkermansia	*Akkermansia muciniphila*

spp, one predominant part of the gut microbiota, has been reported to be essential in maintaining good health. They have been associated with therapeutic effects on intestinal and systemic health, probiotic ability, and the ability to counteract the effects of inflammation and other dysfunctions of the gut.[28] *Lactobacillus* spp, known for their lactic acid-producing ability are commonly used as probiotics and can be found in different parts of the body including the gut, female reproductive system, and so forth.[29] The effects of these microorganisms include colonization resistance against potential disease-causing bacteria.[30] A study by Mu et al.[31] outlined several benefits of *L. reuteri* and these included antimicrobial activity, inhibition of pathogenic activity, anti-inflammatory effects, and so forth. *Bifidobacteria* spp, are also an important group of the gut microflora and have been associated with immune system stimulation and the production of short-chain fatty acids.[32] The examples of some species that make up the gut microflora share similar effects, showing their ability to influence chemical processes that occur and molecules the function within the body, immunity, pathogen-colonization resistance and thus, the normal functioning of the body.[29]

THE ROLE OF GUT MICROBIOTA IN CARDIOVASCULAR DISEASES

Gut microbiota is essential for maintaining the uptake of essential nutrients and immune responses, and it can influence how the host responds to pathologic conditions.[16,33–35] Alterations in gut microbiota can have detrimental effects on human health and studies have reported several of these effects including dysfunctions in the intestinal barrier and systemic health, cognitive impairment, COVID-19 severity, and heart disease.[36–39] Cardiovascular diseases (CVDs) are a leading cause of death worldwide and has high morbidity and mortality rates[19,35,40,41] Multiple risk factors contribute to the development of CVDs such as obesity, diabetes, hypertension, and dyslipidaemia that result in permanent damage to the cardiovascular system.[35,40,42] Among these detrimental risk factors, abnormal immune regulation, and metabolic disorders play an important role in the progression of CVDs.[19,40] In recent years, studies have found that alterations in the gut microbiome have been linked to CVDs.[16,33,34] Atherosclerosis is a chronic inflammatory disease that is considered a key factor in the onset and progression of CVD.[33,34,40,43] Several studies have highlighted the presence of an altered gut microbiota in patients with atherosclerosis and coronary artery disease.[42,44,45] Bacterial DNA present in atherosclerotic plaques correlates with the presence of number of white blood cells in atherosclerosis plaques[43] and microbial species differ significantly between atherosclerotic patients and healthy individuals.[46,47]

Given the instrumental role of gut microbiota in CVDs, the opposite is also true, which is that it could serve as a potential target for antihypertensive therapies.[19] Here, the importance of a particular metabolite produced by gut microbiota, trimethylamine-N-oxide (TMAO) has been highlighted.[40,43] TMAO is produced through the digestion of choline, phosphatidylcholine, and carnitine, found in most animal products and a few plant products.[19,34,48,49] The increase of TMAO strongly influences multiple risk factors for CVD, such as hypercholesterolemia and hypertension.[19,34,42,49] Increased TMAO also reduces cholesterol clearance from the body and associates with the production of atherosclerotic plaques[19,50] (Table 2). Similarly, a strong association has been found between hypertension and TMAO production, data from a meta-analysis demonstrate that individuals with higher TMAO are more likely to develop hypertension[51,52] (Table 2). Therefore, TMAO levels have considerable biomarker potential CVDs.[40,53] However, much remain to be better understood regarding the full extent of gut microbiota's involvement in the development of treatment of CVDs.[54]

THE ROLE OF GUT MICROBIOTA IN PULMONARY HYPERTENSION (PH)

PH is defined as a mean pulmonary artery pressure ≥20 mmHg, when diagnosed with right heart catheterization.[60,61] Characteristic features include sustained vasoconstriction, vascular remodeling, and thrombosis formation.[62] Pathologic triggers are believed to prime cells in the pulmonary arterioles to excessive proliferation that leads to narrowing and obliteration of the vessel lumen, and thus, elevated pulmonary artery pressure.[63] There is a growing body of evidence from both preclinical and clinical studies demonstrating that gut microbiota and its by-products play a role in the development of PH.[11,64,65] The full process is complex and poorly understood, but an important finding is that microorganisms of the gut are also present in the lungs,[10] and these include *Bacteroidetes* and *Firmicutes*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*.[66]

The *Firmicutes* to *Bacteroidetes* (F/B) ratio in PH

Anaerobic *Firmicutes and Bacteroidetes* make up >90% of the total bacterial species in healthy individuals.[56]

TABLE 2 A summary of key studies that highlights gut microbiota signatures in the context of cardiovascular disease and PH.

Gut microbiota signature	Model	Findings	References
Cardiovascular diseases			
	Humans	The increase in TMAO strongly influences multiple risk factors for cardiovascular diseases, such as hypercholesterolemia and hypertension	[19, 34, 42, 49]
		Increased TMAO also reduces cholesterol clearance from the body and associates with the production of atherosclerotic plaques	[19, 50]
		A strong association has been found between hypertension and TMAO production, data from a meta-analysis demonstrate that individuals with higher TMAO are more likely to develop hypertension	[51, 52]
PH	*Monocrotaline-induced rats*	Elevated *Firmicutes-Bacteroidetes*	[55]
	Hypoxia plus SU5416 treated rats	Three-fold increase in *Firmicutes*-to-*Bacteroidetes* ratio. Acetate and acetate-producing bacteria to be reduced in the serum of PH rats.	[56]
	ACE2 knock-in PH mice	Imbalances between beneficial bacteria such as Bacteroids and SCFAs-producing bacteria, and potential pathogenic bacteria such as TMA/TMAO-associated bacteria, are in parallel with PAH, with functional changes of microbiome	[55]
	Humans	Increased *Firmicutes-Bacteroidetes* ratio in PH is due to a decrease in *Bacteriodetes* as well as *Firmicutes* enrichment	[57]
		No change demonstrated in alpha-microbiota diversity	[55, 56]
	Humans	Reduced alpha-diversity	[57–59]
	Humans	Reduction in genes responsible for generating the SCFA metabolite, propionate	[59]

Abbreviations: SCFA, short chain fatty-acids; TMAO, trimethylamine-N-oxide.

Characteristic findings of dysbiosis is an increased *Firmicutes-Bacteroidetes* ratio, reduction in short chain fatty-acids (SCFA) producing bacteria and decreases in acetate- and butyrate producing bacteria as well as increase in lactate producing bacteria[56] (Table 2). An elevated *Firmicutes-Bacteroidetes* ratio, especially due to reduced *Bacteroidetes* have been shown in both pre-clinical and clinical studies to be a feature of altered gut microbiota in PH. These findings were echoed by Sharma et al.[55] who also demonstrated that an increased *Firmicutes-Bacteroidetes* ratio in monocrotaline induced PH (Table 2). Callejo and colleagues demonstrated in a hypoxia plus SU5416 treated rats an almost four-fold decrease in the *Bacteroidetes* population in PH induced rats (Table 2). Zhang et al.[57] demonstrated that an increased *Firmicutes-Bacteroidetes* ratio in PH patients is due to a decrease in *Bacteriodetes* as well as *Firmicutes* enrichment (Table 2). Furthermore, although there is no change demonstrated in alpha-microbiota diversity in PH animal models,[55,56] both Li et al.[58] Kim et al.[59] and Zhang et al.[57] demonstrated a reduced alpha-diversity in PH patients.

The reduction in SCFA producing bacteria and TMAO in PH

Reduction in SCFA producing bacteria contributes to increased gut leakiness and inflammation through the release and circulation of for example, acetate.[67] Cajello et al.[56] found acetate and acetate-producing bacteria to be reduced in the serum of PH rats (Table 2). Kim et al.[59] also found a significant reduction in genes responsible for generating the SCFA metabolite, propionate, in patients with PH, thereby confirming the potential role of gut microbiota in the pathogenesis of PH in humans (Table 2). On the other hand, various bacterial communities are associated with TMA/TMAO production, including *Coriobacteriales* which is significantly increased in PH[6] (Table 2).[7] TMAO promotes the

proliferation and migration of pulmonary artery smooth muscle cells, by upregulating inflammatory factors.[11,68] Endothelial dysfunction has also been shown to be influenced by TMAO via inflammatory pathways that lead to endothelial hyperpermeability.[58,69] Furthermore, TMAO promotes the production of reactive oxygen species that adds to the impairment of endothelial function.[58,70] Poor prognosis and increased severity of disease were associated with increased TMAO in PH (Li and colleagues).

Streptococcus as part of the microbiota, and its role in PH

Kim et al.[59] have demonstrated an association between *Streptococcus* and PH in humans, while animal studies have shown depleted *Streptococcus* levels.[55] Interestingly, *Streptococcus* has been implicated in the activation of a pro-survival kinase pathway mediated via ERK and PI3K.[71] These kinases are involved in the pathogenesis of PH through the induction and proliferation of pulmonary smooth muscle cells.[72] *Streptococcus* also promote serotonin production[73] which has previously been implicated in the smooth muscle cell contraction and proliferation contribution to PH pathogenesis.

CROSSTALK BETWEEN MICROBIOTA AND LUNGS IN PH VIA INFLAMMATORY MEDIATORS

Crosstalk between the lung and gut, also termed the *gut-lung axis*, comprises a key mechanism through which the gut microbiota influences the pathogenesis of respiratory diseases.[66,74,75] The gut microbiota plays an important role in the regulation of the immune system,[76,77] specifically, systemic and lung immunity, via the regulation of T-cell differentiation, the migration and apoptosis of immune cells, and the activation of toll-like receptor signaling.[78] The microbiota play a role in modulating immune function in the prevention and progression of chronic respiratory diseases.[79,80] Therefore, there is a crosstalk between the lungs and gut microbiota. This cross-talk is important as it can influence the pathogenesis and treatment of lung diseases,[79–83] and furthermore, the amount and function of immune cells activated through the gut-lung crosstalk, influences the pharmacokinetics and bioavailability of therapeutics for lung diseases.[84] Also important is that when there is dysbiosis of the gut microbiota, clearance of lung macrophages becomes compromised in diseases like for example, lung tuberculosis, leading to an imbalance in cytokine production, and the activation and migration of T and B cells become impaired.[83] When the gut microbiota is in a state of equilibrium, there is immune function homeostasis which equates to homeostatic cytokine production, correct clearance of lung macrophages, and the proper activation and migration of T and B cells.[83] The bulk of studies therefore confirm the crosstalk between gut and lungs, and the instrumental roles of gut microbiota in the pathogenesis of lung diseases.[80]

Although the mature respiratory and gastrointestinal tracts are different in several ways, one must recall that they originate from the same embryonic structure and thus, they do also display similarities in structure and microbial colonization.[85,86] Both derive from the endoderm and consist of columnar epithelial cells with projections (microvilli in the gut and cilia in the respiratory tract) that serve as a physical barrier as well as forming part of the immune system in collaboration with lymphoid tissue, allowing both organs to influence each other's immune responses and creating somewhat of an immunological relationship.[64] However, impaired gut permeability and systemic migration of gut microbiota is believed to be associated with systemic inflammatory responses that may act as a trigger factor for PH pathogenesis.[87] The gut-lung crosstalk[87] occurs in both directions (to and fro), and is achieved when cytokines, endotoxins, hormones, and metabolites are circulated between the gut and the lungs[88] This happens, for example, during lung inflammation, when inflammatory mediators (interleukin-6, interferon-gamma, tumor necrosis factor-alpha) are circulated to the gut, where lymphocytes are activated and transported back to the lungs[89] Thus, the gut microbiota is closely linked with the pulmonary system to provide protection in the presence of lung pathology.

However, one must keep in mind that the crosstalk is a homeostatic process, and an imbalance (abnormal inflammatory processes)[22,66] may also contribute to PH. Furthermore, inflammatory, and immunological processes are key to the pathogenesis of PH.[16,90,91] In experimental PH, rats display dysbiosis, which excessively increases their *Firmicutes*-to-*Bacteroidetes* ratio and reduces their acetate-producing bacterial genera.[92] This likely happens when gut dysbiosis increases gut permeability and allows the transport of gut bacteria and bacterial endotoxin to the pulmonary vasculature where it could trigger PH[92,93] (Figure 1). Some investigators have suggested that these abnormal inflammatory processes may contribute to the development of PH,[92] and this interaction between

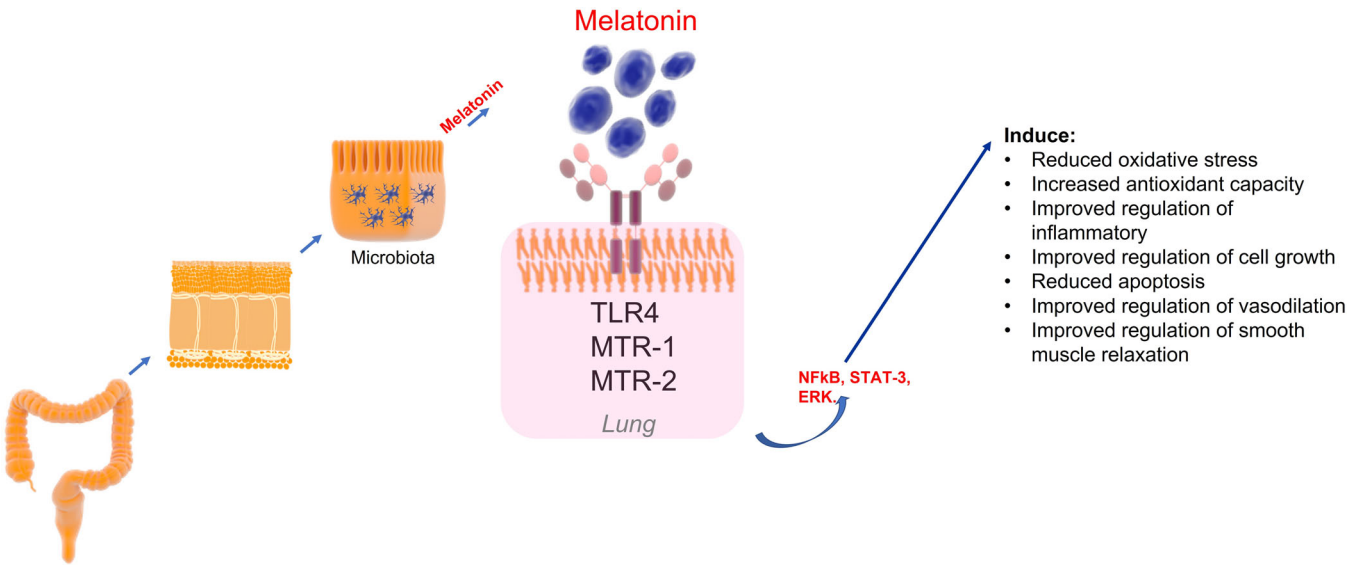

FIGURE 1 Depicts how the gut microbiota produce and release certain factors (inflammatory, genetic, and melatonin) as crosstalk between the gut and the lungs.

the gut and lungs occur via lung-gut crosstalk (Figure 1). The crosstalk is further mediated by bacterial lipopolysaccharides and interleukin-6 that are circulated from the gut to the lungs.[92]

CROSSTALK BETWEEN MICROBIOTA AND LUNGS IN PH VIA GENETIC FACTORS

Abnormal gut microbiota early in life, is linked to the development of lung diseases later in life.[94–96] This has been ascribed to the insufficient maturation of gut microbiota, the excessive use of antibiotics[87,97,98] a genetically inherent risk.[94–96] For example, susceptibility to autoimmune diseases is under strong genetic control by class-II human leukocyte antigen (HLA) allele combinations[99] Core microbiome and beta diversity (i.e., the ratio between regional and local species diversity)[100] differs with HLA risk group and genotype, and in addition, there is also the presence of inherent protective HLA haplotypes that play a role.[99] These are important factors to consider, as an inherent genetic risk can play a role in determining which patients are more susceptible to diseases. This may have relevance to PH too, HLA haplotypes (HLA risk) have been linked with the pathogenesis of PH in patients, and based on the work of Russel and colleagues,[100] gut microbiota are involved in different HLA risk groups (Figure 1). This is an important form of crosstalk between gut and lungs, and surely plays a role in PH pathogenesis.

CROSSTALK BETWEEN MICROBIOTA AND LUNGS IN PH VIA MELATONIN

Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone produced by the pineal gland in the vertebrate brain, but also occurs in fruits and vegetables.[101,102] It is present in all bodily cells and play a role in regulation the circadian rhythm, and can induce anti-inflammatory, anticancer, antioxidant, and vasodilatory effects[103] In the brain, melatonin is mainly produced at night in response to changes in environmental light which leads to serotonin-N-acetyltransferase activity increasing significantly.[104–107] Serotonin-N-acetyltransferase is responsible for converting 5-hydroxytryptamine into N-acetylserotonin, is methylated by acetylserotonin-O-methyltransferase to form melatonin.[108] These moieties are also modulated by noradrenergic and neuropeptidergic projections to the pineal gland as well as light information that is transmitted from the retina to the pineal gland via the suprachiasmatic nucleus in the hypothalamus. In humans, melatonin secretion begins shortly after sunset and peaks in the middle of the night. Although melatonin is the main hormone produced by the pineal gland, extra-pineal sources include the retina, bone marrow cells, platelets, skin, lymphocytes, cerebellum, and especially the gut, among other.[109] The concentration of melatonin in gut can be as high as 10–100 higher than in plasma and up to 400 times higher than in the pineal gland.[108,110,111] The most well-known molecular pathways through which

melatonin acts, is the activation of G-protein coupled membrane receptors, MT1 (or Mel1a or MTNR1A) and MT2 (or Mel1b or MTNR1B).[108,112,113] MT1 melatonin receptor activation inhibits cAMP in target cells and is associated with reproductive and metabolic functions, as well as vasoconstriction.[104,114] On the other hand, MT2 receptor activation leads to cAMP and cGMP inhibition, plays a role in regulating circadian rhythms and dopamine release in the retina, and also contributes to vasodilation.[113] Melatonin interact with the cardiopulmonary system via various signaling pathways.[115–118] It does so by directly exerting its function over a receptor dependant pathway including membrane receptors type 1 (MT1) and type 2 (MT2) as well as the retinoid-related orphan nuclear receptors RZR and RORα.[116,119] It also exerts its beneficial effects indirectly as a free radical scavenger by protecting cells against oxidative stress, reducing the generation of free radical and reactive oxygen species.[120–122] Melatonin regulates blood pressure by centrally modulating the baroreflex set point, and the sympathetic and parasympathetic vascular tone.[123]

The involvement of melatonin in PH

In patients with PH, levels of melatonin are lower than in healthy patients and this negatively correlates with an increase in cytokine levels.[62] Also, lower levels of melatonin correlate with a worse long-term survival rate of PAH patients.[124] Several animal studies have provided evidence advocating the beneficial role of endogenous melatonin. Hung et al.[125] outlined that melatonin can improve vascular resistance and oxidative injury in hypoxia-induced PH. By using a rat model, they demonstrated how right ventricular systolic pressure, vascular remodeling, markers of oxidative stress and inflammation, TNF-alpha, and malondialdehyde, were significantly decreased when chronic hypoxic rats were given pharmacological doses of melatonin. They also observed that the nitric oxide bioavailability has increased.[125] Torres et al.[126] reported similar results, including enhanced pulmonary vascular function in new-born sheep with PH. An in vitro model showed attenuated proliferation and inflammation in pulmonary arterial muscle cells in the presence of melatonin while a rat model showed improvements in right ventricular systolic pressure and pulmonary vascular remodeling.[127] Zhang and colleagues demonstrate how melatonin significantly ameliorated hypoxia-induced thickness of the pulmonary artery wall and improved the remodeling of blood vessels. They also demonstrated that PH is associated with decreased serum melatonin levels.[62] While decreased levels of melatonin were linked to poor prognosis and increased mortality, Cai et al.[124] reported that at the time of diagnosis, serum melatonin levels can be used to anticipate the clinical outcome of PH. Maarman and colleagues in their monocrotaline rat model demonstrated how melatonin therapy at both therapeutic concentration and at concentration as found in food protects against PH by reducing oxidative stress, reduced right ventricular hypertrophy, improving right ventricular function and cardiac interstitial fibrosis. They however noted that the dose of melatonin required for an equivalent effect in humans would be substantially higher than what was the dose used in clinical studies at the time.[128] In concert, these studies demonstrate the key role of melatonin in PH. However, there is a shortage of literature that describes melatonin as a key player in gut-lung crosstalk, and how it can potentially modulate the pathogenesis or treatment of PH.

The roles of melatonin in promoting gut microbiota reprogramming

Melatonin contributes to improved metabolism of lipids[1] and remodeling of the gut microbiota composition in both animals and humans.[1,2] Yin et al.[129] demonstrated in a gut microbiota transplant mouse model that was subjected to a high fat diet, displayed an accumulation of intestinal lipids as well as dysbiosis. In this study, melatonin reduced lipid concentration and the reversal of dysbiosis. The *Firmicutes-Bacteroidetes* ratio was also significantly increased and improved synthesis of acetic acid upon supplementation with melatonin. These findings therefore support the important role melatonin plays in reprogramming of the gut microbiota.[129] It their study, Lui et al.[130] investigated the role of melatonin in alleviating dysbiosis caused by aflatoxin B1 and its effects on the intestinal/liver axis, they found that the alterations due to aflatoxin B1 exposure to be corrected by melatonin administration. Melatonin reduced the relative abundance of *Firmicutes* and the *Firmicutes-Bacteroides* ratio and elevated that of *Bacteroides* at the phylum level of the taxonomic units, while also decreasing the relative abundance of *Clostridiales* and *Lactobacillales*. Aflatoxin B1 exposure resulted in elevations in the relative abundance of *Desulfovibrio*, *Clostridium-XIVa*, and *Lactobacillus* at the genus level, but upon exposure to melatonin these changes were reversed. It was concluded that the dysbiosis in the gut microbiota due to AFB1 was successfully reprogrammed by melatonin.[130]

Moreover, the gut microbiota can produce and release serotonin[131] and tryptophan,[132] which are precursors for the formation of melatonin. Melatonin concentrations are 400 times higher in the gut than in the pineal gland.[133,134]

The gut has toll-like receptors, which are molecular patterns of antigens whose activation can stimulate innate immunity to destroy pathogens.[135] During the early postnatal stage, the body reduces the expression of toll-like receptors in the gut, to promote healthy gut microbiota.[136] In turn, the gut microbiota can trigger toll-like receptor signaling to suppress inflammatory responses that make it possible for these good microorganisms to survive in the gut.[136,137] This is important, because the gut expresses several receptors on its surface, including the toll-like receptors[137] and melatonin receptors 1, 2, and 3.[138] These receptors can bind melatonin[139] that resides within the gut and the lungs.[133,134] To this end, Kim et al.[140] showed that improved sensing of bad bacteria through toll-like receptor-4 and the regulation of bacteria through altered goblet cells and antimicrobial peptides in microbiota, is involved in the anti-colitic effects of melatonin. Melatonin-induced activation of these receptors stimulates the expression and release of NFkB, STAT-3 and ERK[141] which are known role players in PH pathogenesis (Figure 1).

GUT-LUNG CROSSTALK IN PH VIA MELATONIN: THE MAIN HYPOTHESIS

Melatonin levels can be reduced by stress,[142] smoking,[143] or shift work,[144] where reduced melatonin are considered pathologic. Furthermore, low serum levels of melatonin associates with PH. Therefore, it is likely that dysbiosis from psychological stress or other factors, may reduce levels of melatonin and contribute to the development of PH (Figure 2). Thus, dysbiosis reduces melatonin signaling (via TLR4, MTR-1, MTR-2) and secretion in and from the gut, and less melatonin reaches the lung, this limits its protective effects and predisposes to the development of PH. Considering that exogenous melatonin can improve experimental PH,[60,128] it is of interest to delineate the roles of melatonin as a crosstalk agent between the gut microbiota and the lungs. Patients and animal models of PH display impaired gut microbiota,[59,145] and it makes one wonder whether this is also associated with impaired melatonin crosstalk[92] Furthermore, would the latter contribute to a predisposition to develop PH? Further investigations are needed to better understand the roles and involvement of melatonin in the crosstalk between gut and lungs. Future studies could investigate the levels of melatonin in the gut and lungs, in appropriate models of PH,[146] as this may provide useful information regarding its involvement in the crosstalk between the gut and lungs. Using radioactive labeling of melatonin ($^{[101]}$I-2-iodomelatonin)[147] could help to trace the melatonin crosstalk, over time. For example, in a rat model of PH one could measure melatonin levels in the gut, lungs and circulation before PH is induced, and measure it over a period of time. In this instance, one might be able to see how melatonin levels change in the

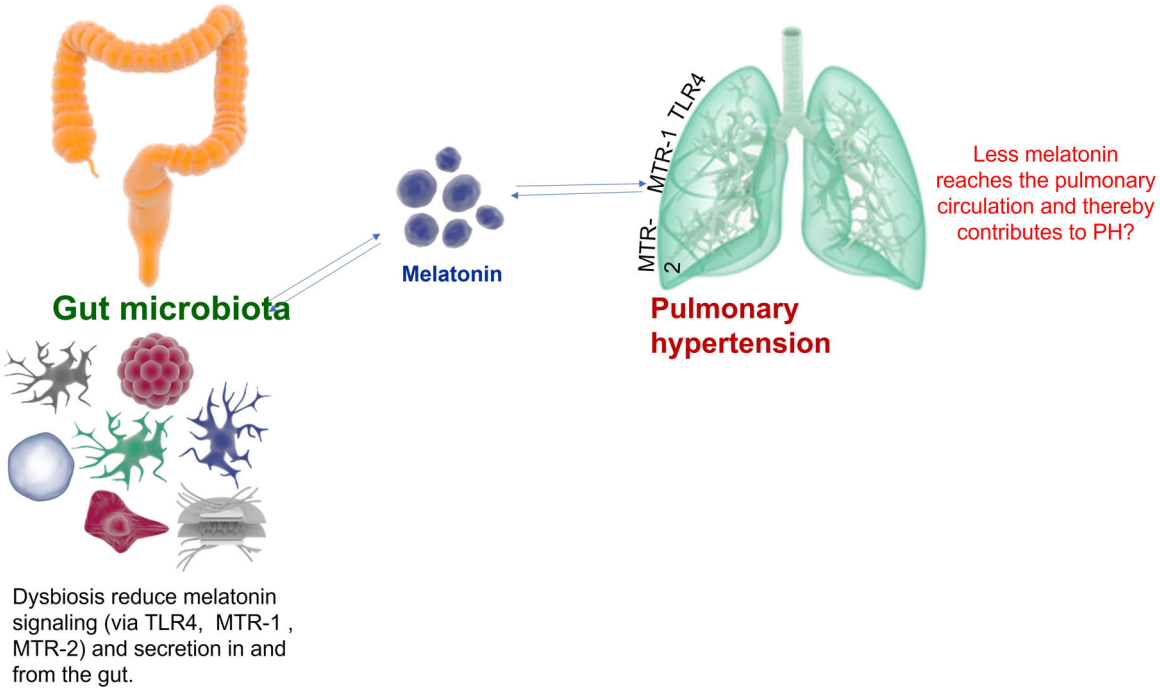

FIGURE 2 Depicts how the gut microbiota produces and releases melatonin as a crosstalk between the gut and the lungs. Melatonin can activate and regulate signaling pathways that could induce processes that may underpin the development of PH.

gut, lungs, and circulation over time. A decrease melatonin levels in the gut and an increase in the circulation and lungs, may suggest a crosstalk effect, or movement of melatonin from gut to lungs during PH. This might mean that (1) a lack of melatonin contributes to PH, (2) that increased levels is a compensating effect for the existing lung damage in PH, and (3) may confirm the melatonin crosstalk in PH. However, whether these investigations are performed in animal models or patients, it is important to keep in mind that time of day and light exposure may be confounding factors that influence the levels of melatonin that is measured.[147]

CONCLUSIONS

It is plausible that melatonin and its receptors in both the gut microbiota and the lungs may provide a relatively novel crosstalk between these two biological sites. Studies can investigate whether microbiota and melatonin genes play a role in the epigenetic aspects of PH, as dysbiosis (caused by epigenetic or environmental factors) could reduce melatonin levels and signaling or subcellular concentrations in the gut or reduce melatonin. This may help to explore the role of melatonin in the crosstalk between the gut microbiota and the lungs during PH. This area of research is largely unexplored in the context of PH and may present a good opportunity for the discovery of novel therapeutic targets or pathologic role players in PH pathogenesis.

AUTHOR CONTRIBUTIONS

All authors meet the criteria to qualify as authors, have contributed sufficiently to the manuscript, and have seen/approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICS STATEMENT

No ethical requirements were relevant for this paper.

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