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Microbiota and microRNAs in lung diseases: mutual influence and role insights

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Abstract. – Trillions of microbial cells colonize human body both internally and externally. The prevalent amount of these reside in the gastrointestinal tract (gut microbiome). Gut microflora support the transformation of food nutrients. The products of this modification processes both modulate gastro-intestinal immunity, and influence other organs such as lung and brain. Recently, it was reported the role of micro-RNAs (miRNAs) as regulators in different pathways of the innate and/or adaptive immune responses. Latest studies discussed the aptitude of probiotics strains to balance the host immune response at a post-transcriptional level by controlling miRNAs expression. We speculated a model of lung immune regulation driven by the axis microbiota-microRNAs, involving asthma, acute injury, cancer and COPD. Based on this axis, we propose a novel approach based on the modification of microRNAs expression centered not exclusively on antagomiRs but also on microbiota modification in order to further potentiate their therapeutic effects.

Key Words:
Microbiota, MicroRNAs, Lung, Cytokines, Inflammation, Cancer.

Introduction

Nearly 40 trillion microbial cells are thought to be part of the human body both internally and externally. The prevalent amount of these cells reside in the gastrointestinal tract and is known as "the gut microbiome". During last decade, the alteration of the set of gut microbial cells (dysbiosis) has been linked to several disorders[1]. Gut microflora support the transformation of food nutrients which are normally indigestible. The products of this modification processes both modulate gastro-intestinal immunity, and influence other organs as lung and brain. Gut dysbiosis has been associated to several lung diseases, like allergy, asthma and cystic fibrosis. The Gut-Lung axis, a bi-directional interchange between the two organs, is translated as intestinal disturbances reported in lung diseases[2].

The therapeutic administration of probiotic bacteria was demonstrated to have positive outcomes for the health: primarily increasing immune homeostasis by modifying microbial equilibrium and secondary balancing the immune system[3-5]. Although the protecting ability of probiotics is well-known, only little is known about their action in a viable or non-viable state. Dendritic cells (DCs), being antigen presenting cells (APCs), have a key function in the individuation and selection of detrimental pathogens between commensal bacteria and probiotics[6]. This identification is achieved by Toll-like receptors (TLR). Recent papers focused on the downstream after the activation of the complex after TLR recognition; this key point regulates the outcome of probiotics induced-immune response. Specfic phyla in viable and non-viable condition expressly guide the evolution of DCs and priming T cells[5,7]. For example, *in vitro* studies verified that lactobacilli are capable of priming DCs to stimulate the development of Treg cells[8].

Probiotics act as anti-inflammatory supplementation and contribute in preserving human health by reducing the risk of accumulation of inflammatory molecule,s such as reactive oxygen species (ROS)[9].

In terms of importance of bacterial induction of inflammation an example is represented by Lipopolysaccharide (LPS). It is a focal part of

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Gram negative bacteria, and is thought as one of the main ligands of toll like receptor (TLR)-4. It has been observed that signaling through TLR-4 boosts the inflammatory process by triggering the nuclear factor (NF)-kB[10]. Its transcription factor stimulates the expression of pro-inflammatory cytokines, apoptosis and consequent progression of the chronic inflammatory diseases[11]. Several years ago it has been reported[12] that *Lactobacillus acidophilus* is able to reduce the development of such inflammatory status by acting on the gastrointestinal barrier and on macrophages. Moreover, the weight of probiotics in the equilibrium between T helper (Th) 1 and Th2 responses has been emphasized through their balancing properties on the production of pro-inflammatory and anti-inflammatory cytokines[13]. Additionally, it was reported that probiotics are capable of modifing the genes induced during inflammation and thus are able to variate the morphology and target of the cells[14].

Moreover, multiple lines of evidence have highlighted flora as drivers of oncogenesis and as targets of tumour progression in several neoplastic types[15-17].

More recently, the role of micro-RNAs (miRNAs) as regulators of different pathways of the innate and/or adaptive immune responses has been reported[18]. Micro-RNA (miRNA) represent a great family of small non- condign ribonucleic acid molecules (RNA). Each miRNAs could bind to several different target mRNAs leading to play numerous biological functions. In the last few years, it has been demonstrated the correlation between alteration of MiRNA and several inflammatory diseases, but also, in immune and autoimmune diseases and cancer[19]. Recent studies discussed the aptitude of probiotics strains on balancing the host immune response at the post-transcriptional level by controlling miRNAs expression. To date, no studies have investigated the relationship between modulation of miRNAs by probiotics in lung diseases. In this paper we reported how immune regulation could be influenced by the axis microbiota-microRNAs in lung pathologies.

Search Strategy and Selection Criteria

We searched Medline and PubMed for original articles observing the correlation between microbiota composition and microRNAs expression which could have a role in lung pathophysiology published in English between Jan 1, 1963, and Apr 1, 2020, using as terms the name of the most representative phyla (i.e., "Lactobacillus", "Bifidobacterium", "Akkermansia", etc.) and the words "microRNA", "miRNA", or "miR-". Relevant articles published between 1963 and 2020 were identified through searches in the authors' personal files, in Google Scholar, and Springer Online Archives Collection. Articles generated by these searches, and relevant references cited in those articles, were reviewed. Articles published in English were included.

Results

We sorted a list as shown in Table I.

Bifidobacterium Bifidum (BB)

Three studies were conducted by using BB and evaluating the effects on miRNAs expression. Heydai et al[20] reported that BB administration reduced miR-155 expression[20]. From two other studies by Taibi et al[21,22] it emerged the ability of BB to ameliorate the levels of miR-148a[21,22].

Bifidobacterium Longum (BL)

BL demonstrated its efficacy on reducing the levels of both miR-155 and miR-21a. Unfortunately this data was studied only by Fahmy et al[23] with a single research.

Lactobacillus Acidophilus (LA)

This phylum was far one of the most investigated. Its capacity to reduce the levels of miR-155 was reported by three different authors[20,24,25]. Two studies gave back contrasting results concerning miR-21[24,25]. Mir-146a expression was diminished by LA administration[25] as well as miR-9[25].

Lactobacillus Delbrueckii (LD)

LD was studied by Vahidi et al[26]. They proved that the bacterium was able to reduce both miR-155 and miR-181s levels[26].

Lactobacillus Fermentum (LF)

Rodriguez-Nogales et al[27] were able to investigate LF obtaining interesting results. In the samples analysed, miR-155, miR-223, and miR-150 were downregulated and miR-143 resulted being upregulated[27].

Lactobacillus Gasseri (LG)

No effects were provoked by LG administration on miR-16 whose expression was not modified[28].

Table I. List of the phyla tested in the reviewed experiments and their effects in regulating miRNAs levels and, in turn, immune response in lung diseases.

Phylum	MicroRNA +/-	Molecular target	Lung disease associated to the microRNA	Reference
Bifidobacterium Bifidum	miR-155 -	Asthma/Acute Injury	Heydari et al[20]	Taibi et al[21,22]
Bifidobacterium Bifidum	miR-148a +	tumor suppression; STAT3; ROCK1	NSCLC	
Bifidobacterium Longum	miR-21a -	IL-1beta	Asthma	Fahmy et al[23]
Bifidobacterium Longum	miR-155 -	IL-6	Asthma/Acute Injury	Fahmy et al[23]
Lactobacillus Acidophilus	miR-155 -	KRAS –	Asthma/Acute Injury	Heydari et al[20]
Lactobacillus Acidophilus	miR-155 -	anti-ROS	Asthma/Acute Injury	Kalani et al[24]
Lactobacillus Acidophilus	miR-155 -	IL-17, IL-21, IL-6 and TNF-α improve Th17 response	Asthma/Acute Injury/ Allergy	Wang et al[25]
Lactobacillus Acidophilus	miR-146a -	IL-17, IL-21, IL-6 and TNF-α improve Th17 response	COPD/Asthma/Allergy	Wang et al[25]
Lactobacillus Acidophilus	miR 21 +	Apoptosis	Inflammation, COPD	Kalani et al[24]
Lactobacillus Acidophilus	miR 21 -	IL-17, IL-21, IL-6 and TNF-α improve Th17 response	Inflammation, COPD	Wang et al[25]
Lactobacillus Acidophilus	miR-9 -	IL-17, IL-21, IL-6 and TNF-α improve Th17 response	NSCLC	Wang et al[25]
Lactobacillus Delbrueckii	miR-181a -	Anti-inflammatory function +	Acute Injury	Vahidi et al[26]
Lactobacillus Delbrueckii	miR-155 -	Anti-inflammatory function +	Asthma/Acute Injury	Vahidi et al[26]
Lactobacillus Fermentum	miR-155 -	Anti-inflammatory function +	Asthma/Acute Injury	Rodriguez-Nogales et al[27]
Lactobacillus fermentum	miR-150 -	tumor suppression	NSCLC	Rodriguez-Nogales et al[27]
Lactobacillus fermentum	miR-143 +	cell differentiation; K-ras	NSCLC	Rodriguez-Nogales et al[27]
Lactobacillus fermentum	miR-223 -	STAT3 pro-inflammatory/ tumor suppression	Pulmonary Arterial Hypetension / NSCLC	Rodriguez-Nogales et al[27]
Lactobacillus gasseri	miR-16 =	Inflammation	Asthma	Nishida et al[28]
Lactobacillus Rhamnosus	miR-181a -	Anti-inflammatory function +	Acute Injury	Vahidi et al[26]
Lactobacillus Rhamnosus	miR-155 -	Anti-inflammatory function +	Asthma/Acute Injury	Vahidi et al[26]
Lactobacillus Rhamnosus	miR-155 +	p38 -	Asthma/Acute Injury	Giahi et al[6]
Lactobacillus Rhamnosus	miR-146a -	Immune response –	COPD	Giahi et al[6]
Lactobacillus Salivarius	miR-155 -	Anti-inflammatory function +	Asthma/Acute Injury	Rodriguez-Nogales et al[27]
Lactobacillus Salivarius	miR-223 -	STAT3 pro-inflammatory/ tumor suppression	Pulmonary Arterial Hypetension/NSCLC	Rodriguez-Nogales et al[27]

Lactobacillus Rhamnosus (LR)

LR was studied by two different groups. In one case, and for the first time, gut microbiota integrator based on a Lactobacillus gave contrasting data on miR-155 expression. In fact, in one case it was reduced[26], confirming previous data, in another case it was augmented[6]. LR was able to reduce the levels of both miR-146a[6] and miR-181a[26].

Lactobacillus Salivarius (LS)

LS demonstrated its efficacy in diminishing the values of miR-155 and miR-223 like other Lactobacilli did[27].

MicroRNAs and Lung

Here we listed all the miRNAs resulted being influenced by gut microbiota supplementation specifying their main or potential role in lung diseases.

MiR-9: upregulated in non-small cell lung cancer tissues and correlated with adverse clinical features and unfavorable survival; miR-9 could act either as an oncogene or tumour suppressor differing on the cancer types. MiR-9 knockdown inhibits NSCLC cell invasion and adhesion *in vitro*. Transforming growth factor beta 1 (TGF-β1) is a main regulator in NSCLC metastasis.

TGFβ1 stimulates cell invasion and adhesion. It results blocked by miR-9 knockdown. Thus

miR-9 stimulates NSCLC metastasis and represent an oncogene in NSCLC[29].

MiR-16: it was demonstrated being a stress-responsive microRNAs[30].

MiR-21: it is able to act on endothelial cells by rising the level of nitric oxide (NO) and it's able to decrease apoptosis[31]. MiR-21 levels are supposed to be indicator of immune cell activation. Over-expressed miR-21 in macrophages/monocytes is generally connected to pro-inflammatory factors and stimulated by viruses, bacteria and further molecular patterns and have a vital role in the innate immune process[32,33].

MiR-143: some studies[34,35] demonstrated that miR-143 is essential for repressing proliferation and promoting differentiation. Other authors provided some evidence that miR-143 is effective in suppressing colorectal cancer cell growth through inhibition of KRAS (Kirsten rat sarcoma proto-oncogene) translation[36]. A large number of previous published reports demonstrated that KRAS activation occurs in the early stage of carcinogenesis of lung cancer[37].

MiR-146a: it's expressed after the exprosure to bacterial LPS (lipopolysaccharides) and cytokines in order to set the immune response.

Biologically, miR-146a has been indicated as a marker of innate immune tolerance in the gut, preventing the inflammation-induced epithelial injury. miR-146a increase induce tolerance versus stimulation with LPS, which uses the TLR4-MyD88-AKT/NFκB pathway (Toll-like receptor 4, Myeloid differentiation primary response protein 88, protein kinase B/nuclear factor-kappa B)[38].

MiR-148a: able to suppress NSCLC by acting on ROCK1 (Rho Associated Coiled-Coil Containing Protein Kinase 1)[39,40]. MiR148a stopped cell proliferation and invasion through inhibition of signal transducer and activator of transcription 3 (STAT3)[39].

MiR-150: it is highly represented in NSCLC tissues and it is negatively related with expression of the proapoptotic gene *P53*. *M*iR-150 suppresses the translation of *P53* therefore induce proliferation[41].

MiR-155: influence angiotensin II type 1 receptor (AtR1) and E26 transformation-specific sequence factors 1 (Ets-1)[42,43]. It also diminishes the downstream molecules taking part to acute vascular inflammation such as VCAM-1 (Vascular cell adhesion protein 1), cytokines, chemokines and thus reduce the enrollment of leukocytes[44]. MiR-155 additionally supports NSCLC progression[45].

MiR-223: it regulates PARP-1 (Poly(ADP-Ribose) Polymerase 1) expression in cancer as a consequence of DNA damage[46]. Moreover, downregulation of miR-223 has been shown to mediate mechanical stretch-stimulated proliferation of vascular smooth muscle cells[47]. MiR-223 knockout mice spontaneously develop inflammatory lung pathology with increased inflammatory cell infiltration[48,49]. Additionally, miR-223 downregulation in macrophages activates the signal transducer and activator of transcription 3 (STAT3)[50], a critical transcription factor implicated in inflammation. Hence, miR-223 downregulation is associated to proliferative and inflammatory disorders[51]. MiR-223 acts as a tumor suppressor in lung cancer in several steps of tumorigenesis and evolution[52].

MiR-584: it acts as tumour suppressor in numerous types of cancer[53,54]. In NSCLC, miR-584 results diminished in tissue samples[55] and over-expressed in the plasma of NSCLC patients[56]. MiR-584 controls tumorigenesis and malignant evolution by targeting MTDH (Metadherin) and acting on the PTEN (Phosphatase and tensin homolog)/AKT signalling pathway[57].

Discussion

MicroRNAs due to their ability to activate or de-activate gene expression are considered as potential key player in inflammation and in many diseases. During last years, their importance in lung was analysed by our group and other researchers[58-60]. Recently, gut microbiota was found capable of inducing or suppressing miRNAs in human body[61,62]. Therefore, it emerged that microbiota biodiversity is able to influence diseases onset, development and evolution in several organs. Recently, our group confirmed this progression in a manuscript about microbiota influence on miRNAs in relation to cancer development[63]. In this review we focused on lung diseases. Due to literature results we speculated four microbiota-miRNAs-disease axis involving respectively asthma, acute injury, cancer and COPD as shown in Figure 1.

Asthma

Asthma is probably the most studied disease together with NSCLC due to its emblematic inflammatory status. Several phyla were found capable of modifying microRNAs expression. BB, BL, LA, LD, LF, LR, LS administration was effective in reducing miR-155 levels. This reduction

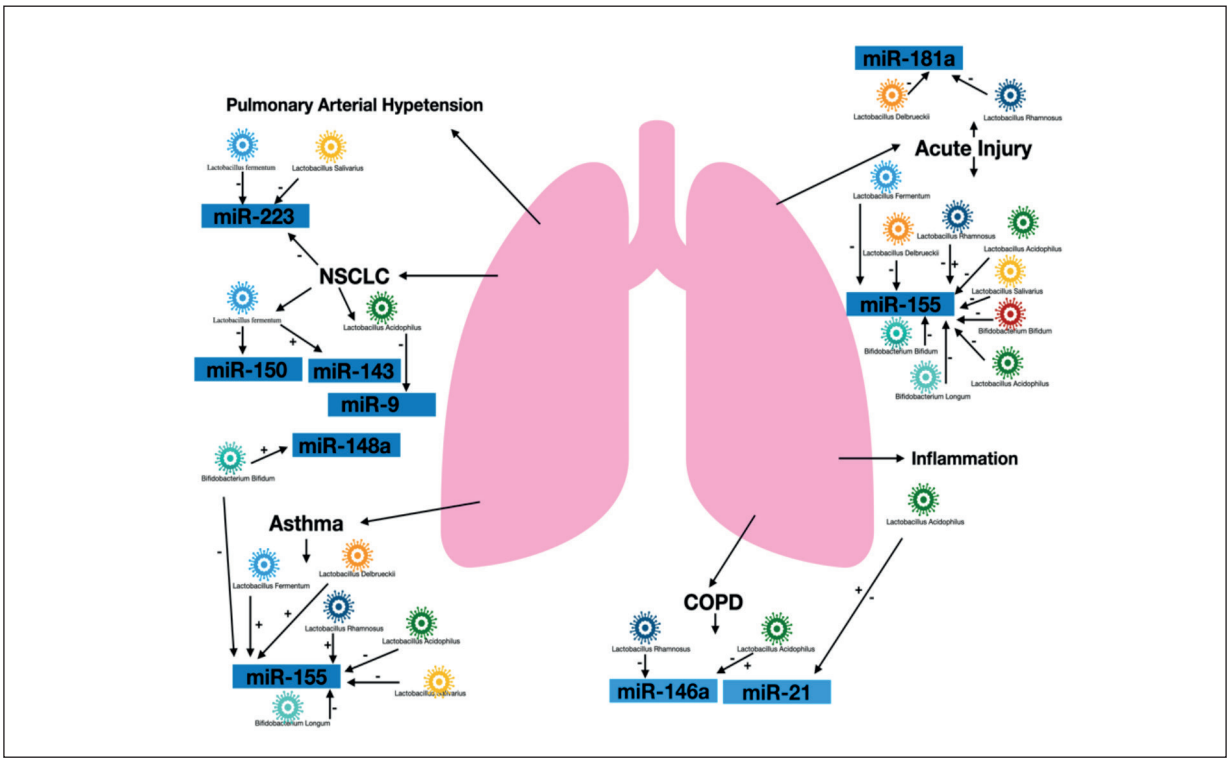

Figure 1. This figure shows the intimate link between gut microbiota biodiversity and microRNAs expression in relation to the specific lung disease.

started an anti-inflammatory process and diminished immune response by the downregulation of KRAS, IL-6, p38, IL-17, IL-21, TNF-a[6,20,23,25-27]; it was also reported a Th17 immune response switch[25]. Another important effect was a higher antioxidant activity[24]. LA and BL downregulated respectively miR-146a and miR-21a targeting the pro-inflammatory cytokines cited above and IL-1b[23,25]. These data highlight an anti-inflammatory trend caused by probiotic bacteria which demonstrated being effective in ameliorating asthma.

Acute Lung Injury

Also acute lung injury resulted being positively influenced by probiotic phyla. BB, BL, LA, LD, LF, LR, LS diminished the levels of miR-155 and miR-181a improving the acute post-damage phase with a significant reduction of KRAS, IL-6, p38, IL-17, IL-21, TNF-a[6,20,23,25-27]. Once more, these microbiota add-ons exerted an anti-inflammatory and anti-ROS function[24].

Cancer

Non small cells lung carcinoma (NSCLC) was the most frequently studied model. BB, LF, LS administration resulted effective in enhancing tumor suppression *via* STAT3 and ROCK1; in fact BB augmented miR-148a and the lactobacilli reduced miR-223 levels[21,22,27]. LA reduced miR-9 which is a pro-neoplastic agent as cited above[25,29]. The anti-cancer effects of probiotics was again confirmed by the downregulation of miR-150 and the over-expression of miR-143 which are known to promote tumor suppression and cell differentiation mediated by K-ras[27].

COPD

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease characterised by a loop release of proinflammatory mediators. They sustain a damage with further mediators recruitment which esitate in a permanent impairment. LA and LR were effective in reducing miR-146a and consequently diminishing the levels of IL-17, IL-21, IL-6 and TNF-α; as expected immune response was also reduced[6,25]. MiR-21, which is considered being a key player in COPD[33] was demonstrated to be influenced by LA administration. According to Kalani et al[24], LA augmented miR-21 levels inducing apoptosis

and promoting COPD worsening; on the other hand, Wang et al[25] reported that LA reduced miR-21 expression with a consequent anti-inflammatory effect[25]. Most data confirmed the beneficial effects of *Lactobacilli* and *Bifidobacteri* integration endorsing once more their potential as supportive therapy.

Immune Balance

Gut microbiota was demonstrated to have a key role in regulating the immune system[64,65]. The papers cited in the above paragraphs highlighted its importance in acute and chronic inflammation. According to literature data, asthma and cancer are the most studied models due to their prevalence and to a clear cytokine involvement. Asthma inflammatory cascade was better defined during last decades and in the mean time it were proposed the first therapies trying to block some vital checkpoints[66-68]. The prognosis of patients affected by severe types of asthma improved at every attempt. However, the use of antibodies against interleukins is not the only therapeutical strategy. Recently, authors focused on targeting single genes by intervening on specific lung and inflammatory microRNAs by using the proper antagomiR[58]. They are a novel group of oligonucleotides, artificial analogs of miRNA, promising as useful tools for the blockade of endogenous miRNA *in vivo*[69]. Although these therapeutic strategies demonstrated being promising, many multi-resistant types of lung inflammatory disorders were also described. Thus, it appeared necessary to raise the level of immune intervention, and many researcher proposed the modification of body microbiota in order to have an ever growing set of anti-inflammatory weapons. For this reason, we collected the most significant papers in the field.

Cancer is not an exception, as it is a hot topic in the field of immune regulation too. In fact, regulating the immunosuppressive neoplastic microenvironment is considered fundamental in cancer immunotherapy[70,71]. The use of immune checkpoint inhibitors (ICIs) became the last frontier in the field of tumour treatments. However, this novel therapeutic approach is not resistance-free. Some recents experiments demonstrated that in a minority of patients the administration of ICIs did not give the same response. Authors identified the problem in the gut microbiome composition. The use of some antibiotics which eliminated some phyla caused a non-response to the ICI[72,73]. Routy et al[73] demonstrated the ability of the microbiota of influencing ICIs response. In fact, by using fecal microbiota transplantation in order to permanently modify mice gut microbiota they amplified ICIs sensitivity[73]. These results highlight how promising could be modifying the quality of body microbiome.

Conclusions

Probiotics *in vitro* studies, as well as *in vivo* administration, let us to better understand their role in organs pathophysiology. The first step was the comprehension of their ability to over and under express some microRNAs. The second step was the understanding of how miRNAs epigenetic could influence the pathogenesis of such important organs. Balancing the immune system is the key to fight against many of the pathologies affecting human body. We focused on lung diseases that often involve acute and chronic inflammation. In fact, every disease analysed resulted having an excessive or a reduced immune system response and activation. Everyday we read and hear about speculation about the potential benefits of probiotics integration. Systematic and bias-free studies are needed in order to explain how they act and how we can potentiate their pathogenetic mechanisms. Recently, the use of antagomiRs represented the most innovative therapeutic proposal to block inflammatory microRNAs. Due to miRNAs peculiarity of modifying gene expression, antagomiRs represented our chance to influence pathologies course. Nevertheless, a novel approach based on the modification of microRNAs expression based not exclusively on antagomiRs but also on probiotics administration could further potentiate their therapeutic effects and represent another ace up our sleeve. The microbiota-miRNAs-lung axis model reflects our point of view of the most recents studies. We hope that it could represent a start for discussing possible future scenarios and for planning innovative research paths.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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