Microbial Regulation of microRNA Expression in the Brain-Gut Axis

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Abstract

The gut microbiome facilitates a consistent transfer of information between the gut and the brain and microRNAs may now represent a key signalling molecule that facilitates this relationship. This review will firstly examine how these small non-coding RNAs influence the gut microbiome, and secondly how the microbiome, when disturbed, may influence miRNA expression in the brain. In addition, we will examine the consequence that microbiome-related changes in miRNA expression have on neurodevelopment, behaviour and cognition. We will also discuss novel data that suggests miRNAs contained in our diet may influence our immune system in a positive manner, offering a further potential pathway for treatment of disorders of the gut-brain axis that are influenced by the microbiome.

Introduction

Recent research continues to expand on the scope of influence of the gut microbiome on the gut-brain axis and to identify an expanding range of microbially-regulated molecular targets in both the gut and the brain. In particular the transcriptional landscape in brain regions of functional importance to stress-related psychiatric disorders is markedly impacted by gut microbiome manipulations. Added to this portfolio is the ability to exert an impact on the expression of microRNAs in discrete brain regions. Moreover, there is a reciprocal impact of host-derived miRNAs locally in the gut. In this review, we provide a brief overview of the fundamentals of miRNAs and then discuss the most important recent advances in this area and their implications for host-microbe interactions.

What do MicroRNAs do?

MicroRNAs (miRNAs) are small, non-coding, single-stranded RNAs that post-transcriptionally regulate the expression of cellular mRNAs that contain miRNA binding sites. Their expression is widespread [1], and they have a broad influence on cellular development and function [2]. Since the discovery of the first miRNA, *lin-4*, in *C. elegans* in 1993 [3], an additional layer of complexity has been added to the regulation of gene expression in many healthy and pathological cell processes. The primary role of miRNAs appears to be post-transcriptional regulation of gene expression and this is achieved through binding to target mRNAs [2]. MiRNAs are regulators of various cellular actions including cell growth, differentiation and death and as a result have shown promising therapeutic potential in treatment of diseases such as cancer and viral infection.

MicroRNA Structure and Target Recognition

In humans, miRNAs can exist in numerous genomic contexts, recognised miRNAs are encoded by introns from coding and non-coding regions, and miRNAs can also be encoded by exonic regions [4]. Many miRNAs located within the same genomic region can form functional clusters that can be transcribed together [5]. miRNAs are directed to their mRNA targets by base pairing [6] with much of this base pairing occurs within the seed region (nucleotides 2-8) of the miRNA (see figure) and the 3'-untranslated region (UTR) of the target mRNAs. In plants, this sequence complementarity is exact whereas in humans, miRNA : mRNA binding is imprecise [7]. The 8 nucleotides within the seed region facilitate the majority of miRNA mediated repression, and are also the binding sites recognised by most target-prediction software packages [8]. The functional importance of miRNAs is emphasised by the fact that each miRNA is predicted to regulate the expression of thousands of protein coding genes, supporting the idea that miRNAs can influence almost every biological process, furthermore, genetic deletion of genes involved in miRNA processing can be fatal [9]. In addition, miRNAs are conserved across species, emphasising the translational benefit of pre-clinical work in this area [10].

Functions of gastrointestinal miRNAs and their impact on the gut microbiota

Through modifying gene expression, their ubiquitous expression and an ability to modulate multiple cell pathways, miRNAs are powerful signalling molecules [10, 11]. Recent studies have confirmed that miRNAs can contribute significantly to cell-cell communication due to the fact that multiple bodily fluids such as blood, plasma, urine, seminal fluid, breast milk, saliva and cerebrospinal fluid contain miRNAs [12]. MiRNAs are generally stable in these fluids and survive extended storage making them ideal candidates as biomarkers of many diseases and conditions. In 2007, Valadi *et al*, was the first to show that vesicles known as “exosomes” were able to transport miRNA from one cell to another and that this miRNA was functional in the recipient cell [13]. Exosomes are small membrane particles (30-100nm) formed from endocytic compartments and released by numerous cell types [14]. Recent studies have promoted a strong interest in exosomes and their cargo, including miRNA, as potential biomarkers of disease and as therapeutic entities that could be used to treat numerous human diseases [15]. This interest should be tempered though by the caveat that recent research has revealed that non-vesicular fractions may also be present in exosomes [16]. miRNAs have the capacity to behave in a manner similar to hormones and can influence the phenotype of recipient cells which may exist a great distance from their origin [17].

The gut provides an ideal habitat for the microbial ecosystem known as the microbiome, containing an abundant population of bacteria, the composition of which is of significant interest in many diseases and to many fields of research [18]. The molecular basis for this host-microbe interaction is of critical importance to understand how the host and its resident microbiome interact and the role of miRNAs in this interaction between host and microbiome is becoming clearer. In 2011, Dalmasso *et al* [19] examined the expression of miRNAs in the colon and ileum of germ-free mice colonized with the microbiota of pathogen-free mice [20]. They found that a number of miRNAs were influenced by colonisation in the ileum (1 increased) and colon (3 increased, 5 decreased) of mice in addition, they noted that altered expression of miRNAs and genes was higher in the colon than the ileum, reflecting a possible influence of microbial load on expression [19, 21]. Further evidence of this bi-directional relationship is seen in humans with colorectal cancer with 76 miRNAs differentially expressed between colorectal tumours and matched adjacent normal tissue, in addition, a number of these miRNAs were found to correlate significantly with specific groups of bacteria or taxa in the tumour microenvironment [22, 23] suggesting that miRNAs may mediate host-microbe interactions and that this interaction represents a key node for intervention in colorectal cancer patients.

Throughout life, diet is known to significantly impact the composition of the microbiome and detrimental changes in diet can lead to detrimental changes in the microbiome [24]. Modulating dietary intake and composition has become a key targeted intervention strategy in regulating the microbiome and understanding how diet can influence host-microbe interactions is an important part of this. While we know that components of the food we eat are important energy sources for the microbes in our gut, less is known about specific interactions between dietary particles and the microbes in our gut. Important work from Zheng *et al* demonstrated that exosome-like nanoparticles (ELNs) contained in ginger and ingested in rodent diet were preferentially taken up by *Lactobacillaceae*. Furthermore, within these ELN particles are miRNAs which were able to target *Lactobacillus rhamnosus* (LGG). Specifically, mdo-miR7267-3p targeted the ycnE gene in LGG which resulted in increased indole-3-carboxaldehyde (I3A which increased interleukin-22 (IL-22) production and improved gut barrier integrity [25].

microRNAs are also a constitutive component of murine and human faeces, derived from the host epithelium, they are detectable in faeces and are essential for maintaining a normal gut microbiota. Their importance in gut physiology is emphasised by depletion of the DICER enzyme in the epithelial cells of mice which display exacerbated colitis and disturbed microbiota [26]. Furthermore, *in-silico* analysis has revealed that faecal miRNAs target bacterial genes with functions such as the glycosylation of mucin and structure of the extracellular matrix [27]. Recent work from our group has found that germ-free mice had decreased expression of miRNAs expressed in the gut epithelium, of interest though, when the microbiome was depleted following antibiotic treatment, expression of these miRNAs was increased in some cases (miR-200a-3p and miR-141-3p) compared to let-7b-3p, and miR-1224-5p which were decreased in antibiotic treated mice. Moreover, their expression correlated with key phyla. This data adds further relevance to the fact the perturbation of the microbiota at specific time points in development is important to the physiological response mediated by non-coding RNAs [28].

Impact of the gut microbiome on brain function and behaviour: a role for miRNAs?

The gut and the brain signal with each other in a manner similar to hormones, with microbes in the gut able to communicate with the brain and the brain able to influence microbes in the gut. The method by which this bi-directional communication takes place still remains to be fully explained but endocrine, neural, metabolic and immune pathways are likely to be involved [18, 29, 30]. One such communication pathway, the vagus nerve, represents a key neural route which facilitates communication between gut microbes that can influence brain mediated behaviours [31] with the stress reducing effects of the commensal bacteria *Lactobacillus rhamnosus* eliminated by severing the vagus nerve [32]. Similarly, the gut can communicate with the brain hormonally, with gut peptides released from enteroendocrine cells modulating appetite [33]. During the prenatal and postnatal periods, the brain and the gut microbiota undergo a period of organisation and assembly that has a long-term impact on the brain and behaviour [34]. The cooperation of many genes and gene networks are required for adequate neurodevelopment [35]. In fact, disruption of these gene networks have been implicated in many neurodevelopmental disorders and psychiatric conditions [36, 37]. Similar to gene-gene interactions and functional networks, the microbiota also plays a key role in neurodevelopment including behaviour and structural development of the brain [38, 39]. Thus, the brain-gut-microbiome axis represents a key communication system that, when disturbed, may be responsible for many immune, metabolic and psychiatric disorders. Given their role in almost all biological processes, it is has recently been proposed that miRNAs are recruited by the gut microbiome to impact on the functions of the brain-gut axis.

The role of miRNA in regulating physiological behaviour continues to be explored, for example, in *Drosophila*, a locus mutation in miR-iab4/iab8 is responsible for the capacity of *Drosophila* to correct itself when turned upside down [40]. In mice, ablation of DICER, a key enzyme in miRNA processing, increases anxiety-like behaviour [41]. Furthermore, the idea that individual miRNAs can influence behaviour comes from data in mice showing that miR-182, possibly through post-transcriptional regulation of actin regulatory proteins (ARPs), can regulate amygdala-dependent memory formation [42]. Similarly, in rats, basal miRNA expression in the reward circuitry of high and low-novelty seeking rats differs depending on the brain region analysed and the genotype of the rats [43] emphasising the precise role that miRNAs can have in specific brain regions. The microbiome regulates behaviours and physiology influenced by miRNAs, particularly stress and anxiety [44] and can also modulate the transcriptional landscape in relevant brain regions. In germ-free mice for example, rodent social interaction was capable of inducing RNA splicing in the amygdala, thus confirming that a functioning microbiome is closely linked with sociability [45] and that RNA can influence behaviours modulated by the gut microbiome.

To further elucidate the role of the microbiome in gut-brain physiology and indeed, miRNA regulated biology, many groups have manipulated the microbiome through depletion with antibiotics or the germ-free mouse. Intriguingly, some of the most characteristic phenotypic behaviours altered in the germ-free mouse are anxiety-like and social behaviours [38, 46, 47], a phenotype that is also commonly observed in other animal models of disrupted microbial colonisation [48]. Microbial depletion using chronic antibiotic exposure results in a different behavioural phenotype in rats, including deficits in spatial memory, decreased visceral sensitivity and increased depressive-like behaviours in the forced swim test [49]. This data suggests that there are different times during development that the brain is vulnerable to manipulation of the gut microbiota although it remains to be fully understood if microbial manipulation of miRNA expression at different times of life may also impact behaviour [49, 50].

The hippocampus is a brain region associated with learning and memory, and in the rat changes in miRNA expression are influenced by exposure to early life stress and interestingly, these changes can be reversed by treatment with antidepressants [51]. Furthermore, inhibition of miR-124 in the hippocampus of mice improved performance in the Morris Water Maze task and also a spontaneous alternation task in a closed elevated plus maze [52], demonstrating the important role specific miRNAs can have on specific behaviours associated with this brain region. Work from our group has focussed on the possibility that the microbiome is needed for miRNA control of gene expression in the brain. We have previously demonstrated that [53] the gut microbiota can control gene expression in the brain through a network of miRNAs and their target mRNAs [54]. Moreover, some of these changes were only present in males, suggesting that expression patterns of miRNAs can be affected by sex. In this study, mir-294-5p was increased in germ-free mice and importantly, expression was decreased upon recolonisation, providing clear evidence that the microbiome can control expression of miRNA in the brain. *In-silico* analysis revealed that mir-294-5p targeted the pathway associated with kynurenine metabolism and that genes associated with this pathway (Kat1, Tdao1) were differentially expressed in germ-free mice [53].

Along with the hippocampus the prefrontal cortex (PFC) and amygdala have also demonstrated susceptibility to modulation by the gut microbiota. Hoban *et al* presented data that suggested that miRNA expression in these key brain regions were dependent on a functional gut microbiota and that this was especially important during key developmental windows [37]. Using germ-free mice, devoid of all microbiota, they found that miRNAs in the PFC and amygdala, are sensitive to the presence of a microbiome, and that upon recolonisation, the expression of some of these miRNAs are normalised, while some are not. In the amygdala, miR-183-5p and miR-182-5p were decreased in germ-free mice and subsequently expression was restored upon recolonisation. These miRNAs have been implicated in the amygdala response to fear and stress [42, 55] and also, miR-183-5p is increased in the circulation of depressed patients undergoing antidepressant treatment [56]. From this study, *in-silico* analysis of predicted target genes of altered miRNAs in the amygdala and PFC revealed a potential function of these miRNAs in neuronal development, axon guidance and neuronal differentiation which further highlights the role of miRNAs in regulating these key pathways.

Future work in this area must focus on the mechanisms underpinning these observations, including what factors drive this selectivity.

Germ-free mice represent one way of interrogating the function of the microbiome and how it influences development. Analysis using qRT-PCR showed that the expression of some miRNAs are also sensitive to the depletion of the microbiota, for example, a significant decrease in miR-206-3p and miR-219a-2-3p and an increase in miR-369-3p in the amygdala of rats exposed to antibiotics. Other miRNAs that were changed in germ-free mice were unchanged in rats treated with antibiotics, suggesting there are different mechanisms involved and that the microbiota in the rat may differ slightly in the miRNAs its microbiome can influence. What is most intriguing about this work is that, the absence of a microbiome from birth effects the expression of miRNA in the brain differently than in rodents who initially have a normal resident microbiome that is subsequently depleted by antibiotics.

CONCLUSION

How the microbiome influences the expression of miRNAs, their target genes and subsequently how miRNAs influence behaviours mediated in the brain remains to be fully understood. The brain-gut axis represents an attractive starting point and much of this evidence has come from work on vagus nerve signalling [57, 58], and short chain fatty acids (SCFAs) that affect the central nervous system indirectly [59]. How miRNAs can induce signalling pathways via either pathway of communication will offer key mechanistic insights into how miRNAs can influence the brain and how they are controlled by what resides in our guts. Once these mechanistic insights are available, the feasibility of therapeutic targeting of the gut microbiome in an effort to regulate brain-gut miRNAs will become clearer. In addition, we expect a clearer picture of the importance of miRNA-microbe interactions to emerge. Cross-kingdom communication between miRNA and the microbiome represents an intriguing relationship to explore, the interaction of pathogenic [60] and commensal [61] bacteria with miRNAs has only begun to be explored and promises to be a fertile area of exploration.

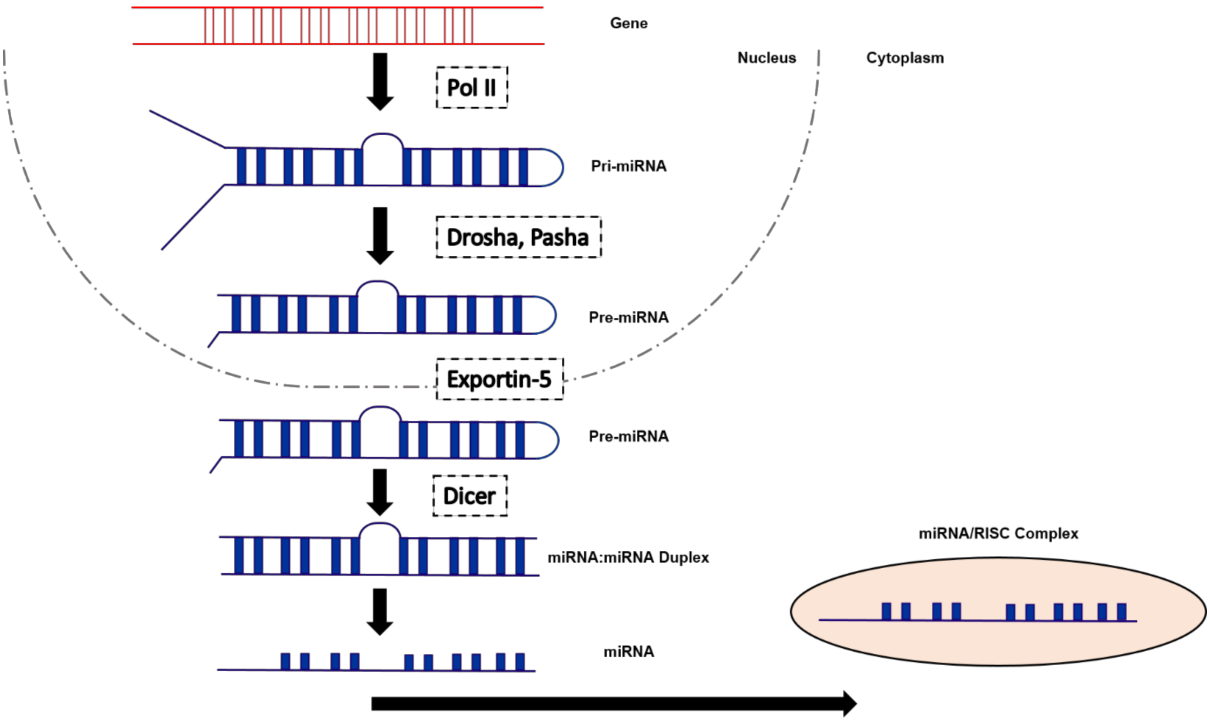

Figure Legends

Figure 1: MicroRNA Processing

The majority of miRNA genes are transcribed by RNA polymerase II, where, in the nucleus, the long primary transcript or the **pri-miRNA**, containing a hairpin region with the mature miRNA sequence within, are processed by two RNASEIII enzymes, drosha and pasha. In the nucleus, the endonuclease DROSHER processes the **pri-miRNA** into a 70-nucleotide stem-loop structure called precursor miRNA (**pre-miRNA).** Following transport to the cytoplasm by Exportin-5; another endonuclease, DICER then processes these transcripts into duplexes of 19-24 nucleotides in length. This duplex is subsequently loaded into the RNA induced silencing complex (RISC) where one strand is selected as mature and the other “passenger” strand is degraded [62].

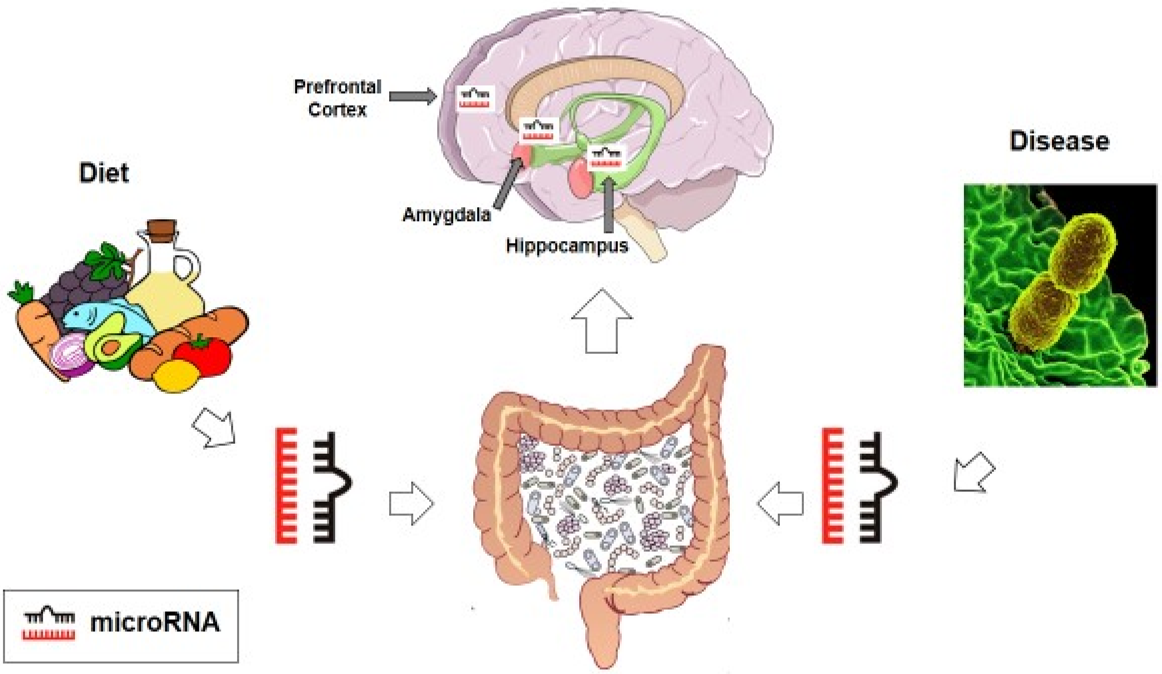

Figure 2

Schematic Representation of the proposed interactions between miRNAs, the gut microbiota and the brain. microRNAs contained within our diet can interact with the gut microbiome, and disease can modify the microbiome via miRNA. In addition, the microbes in our gut can alter the expression of miRNAs within distinct regions of the brain that can influence behaviour.

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