Commandeering the mammalian Ago2 miRNA network: A newly discovered mechanism of helminth immunomodulation

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Abstract:

MicroRNAs (miRNAs) are a class of non-coding RNAs that contribute to a broad range of biological processes through post-transcriptional regulation of gene expression. Helminths exploit this system to target mammalian gene expression, to modulate the host immune response. Recent discoveries have shed new light on the mechanisms involved.

Modulation of Host Immune Responses: A role for Helminth miRNAs

In order to complete their lifecycle, helminths must survive for long periods within their mammalian hosts while safeguarding the fitness of that host. Irrespective of the site of colonisation, infection with every helminth is characteristically associated with a potent Type-2 cell mediated immunity (Th2; [1]). It has been proposed that the Type-2 immune response evolved in mammals as an innate mechanism to regulate inflammation, and to repair damaged tissue. However, over millennia of co-evolution, these same immune mechanisms have been co-opted by helminths to promote host tolerance. As a result, these parasites can establish chronic infections to produce their offspring over extended periods while the tissue damage they cause is readily repaired and excessive inflammation is controlled [1].

Micro(mi)RNAs are short non-coding RNA molecules that regulate the post-transcriptional expression of a large number of genes (**Figure 1**). In this way, miRNAs have central roles in a broad range of biological processes, including the control of immunity [2]. miRNAs bind to their target mRNA 3′ untranslated region (3′UTR) at sites that are complementary to the miRNA 5′ seed region (nucleotides 2–7). This interaction with the specific target gene regulates expression by destabilizing the mRNA and/or inhibiting (and occasionally enhancing) its translation. Comparative analysis of miRNA sequences across species has shown that a number of helminth miRNAs are widely conserved, with some sharing sequence identity with mammalian miRNAs that are known to have an immune regulatory function, which strongly predicts a role for helminth miRNAs in the modulation of host immune responses [2]. Validating this hypothesis, a seminal study reported the modulation of cytokine production in epithelial cells transfected with synthetic mimics of parasite miRNAs, with sequence similarity to host miRNAs [3]. This firmly established parasite-derived miRNAs as a tool to manipulate the behaviour of host cells to benefit the survival of the parasite. However, the cellular mechanism by which the helminth miRNAs were regulating the expression of host genes had not been explored.

Helminth miRNAs must be loaded onto Argonaute proteins to regulate target gene expression. The most important step in the process of target gene regulation is the interaction with effector Argonaute (AGO) proteins. Loading onto an AGO protein facilitates the base-pairing to mRNA targets and mediates the translational repression (**Figure 1**). The paradigm that this organised system of gene regulation could be hijacked and utilised as a mechanism of cross-species communication was first demonstrated in plants, whereby fungal miRNAs bound to plant AGO proteins, consequently silencing mitogen-activated protein kinase genes, to suppress protective immunity [4]. More recently, we have determined that similarly, a conserved miRNA (miR-125b) secreted by the helminth *Fasciola hepatica* is loaded onto the mammalian AGO2 protein within macrophages of infected animals, and acts to regulate expression of pro-inflammatory cytokines [5]. This mechanistic concept is supported by immunoprecipitation of AGO2 loaded with *Schistosoma japonicum* miR-125b after the transfection of macrophages with the parasite miRNA *in vitro* [6]. These observations challenge previous opinion that in order to be functional, helminth miRNAs must be secreted in complex with a parasite-derived AGO protein [3], and instead suggests that miRNAs released by helminths can be processed by a mammalian Dicer and loaded onto host AGO proteins for functionalisation.

It is now of interest to determine whether the sequence of parasite-derived miRNAs dictates the interaction with mammalian host AGO and whether homology to a mammalian miRNA is a requirement for this association. Predictive analysis of miRNAs that are unique to helminths endorses their capacity to also target host genes [7] and *in vitro* analysis with miR-4989-3p, a miRNA that is unique to tapeworms, showed that this was possible, as transfection of macrophages with this miRNA altered the expression of genes associated with the TNF immune signalling pathway [8]. Beyond sequence similarity to conserved mammalian miRNAs, the structure of helminth miRNAs may dictate whether they are processed through the mammalian miRNA network. A near perfect single stem with a terminal loop is required for efficient Dicer processing and loading onto AGO2 [9]. Given that the

large majority of helminths miRNAs (conserved and unique) possess these precursor characteristics, processing by mammalian Dicer could be expected. Independent of miRNA sequence or structure, a recent exploration of miRNA:Ago interactions in *C. elegans* has revealed that miRNA function may be guided by specific cellular requirements [10]. Most miRNAs in this model system exhibited a cell-specific pattern of loading onto AGO, which if transposed to the mammalian miRNA network, suggests an additional layer of regulation of miRNA (host and helminth) functionality.

Do Helminth miRNAs Require Delivery by Extracellular Vesicles to be Functional?

Extracellular vesicles (EVs) are small membrane bound packages released by cells to the extracellular environment to transport proteins, RNAs, lipids, and other molecules and as such are crucial mediators of intracellular communication. Not surprisingly, a number of diverse helminths have been shown to exploit this system and produce their own EVs as a mechanism to deliver their miRNAs to host cells. The observation that conserved miRNAs with identical seed regions to mammalian miRNAs dominate the cargo of helminth EVs supports the hypothesis that this is an adaptation by the parasites to tap into their host's regulatory pathway. Furthermore, while the machinery and components required for EV biogenesis are constitutively expressed across all intra-mammalian life stages, the cargo miRNAs are developmentally regulated [11], most likely to ensure the most appropriate regulation of host gene expression to facilitate different stage-specific requirements by the parasite.

In addition to existing as cargo within EVs, helminth-derived miRNAs have also been detected in serum of infected hosts [12]. The remarkable stability of human extracellular miRNAs in sera has been attributed to their association with AGO proteins. However, rather than simply being a mechanism to protect miRNAs from degradation, it has now been reported that these circulating miRNAs:AGO complexes can interact with gene targets via sequence complementary with the seed region. Furthermore, the assortment of miRNAs loaded onto free AGO2 varies according to

pathological conditions, suggesting that this association represents an active mechanism, to support a flexible response, to ensure appropriate regulation of gene expression. To mediate a functional response these extracellular complexes must be internalised by cells. It has been proposed that this uptake can be mediated by association with EVs or via interaction with surface receptors that have the capacity to bind to AGO2 [13]. Collectively, these observations suggest that miRNAs secreted by helminths into their host's circulation may insert into this system and be fully functional (**Figure 1**). However, further studies will be required to determine whether these miRNAs exist in sufficient quantities required for efficient gene regulation.

Concluding Remarks

As the helminth-derived miRNAs within host immune cells continue to be identified, the extent to which helminths manipulate the mammalian miRNA machinery will be revealed. The evidence to date would imply the widespread incorporation of helminth small RNAs into a broad range of host cells (both immune and non-immune) which presents the opportunity for parasites to regulate numerous biological processes. However, before the mechanisms of cross-species RNA communication is pursued, it is important that the correct bioinformatic approach is used to untangle small RNA sequencing data and distinguish true parasite-derived sequences from those of the host [14]. Using the optimal combination of genome mapping and differential expression, to identify parasite-derived miRNAs interacting with host AGO proteins [5,6], provides an important starting point for future functional studies. These are necessary as many questions remain. Further information regarding the impact of sequence, structure and cellular localisation of helminth miRNAs on the interaction with AGO, as described here, will be critical to fully understand the mechanism of loading. In addition, while most studies focus on the resulting inhibition of host gene expression, another consequence, which has not been widely considered, is that loading of any helminth miRNAs to AGO proteins will alter the stoichiometric levels of AGO. This will reduce the availability of AGO to bind endogenous miRNAs and may indirectly contribute to the overall effect of these helminth miRNAs

on host gene expression. Collectively, solving these questions will ascertain whether interfering with the activities of parasite miRNAs can provide a novel approach to therapeutic control, by preventing their capacity to take control of the host immune response. At first glance, it would seem that the helminths have the upper hand. Targeting the helminth miRNAs with the same sequence, and function, as their host, is not possible as this would likely have a negative effect on the biology of the host. However, if host immunity is also regulated by miRNAs that are unique to helminths these could be targeted for suppression without affecting endogenous host miRNAs. Possible strategies to achieve this could include the use of short LNA antisense or long RNA sponges. Addressing these many considerations will begin to unravel the molecular pathways of this remarkable host-parasite relationship.

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Declaration of Interests

The authors declare no competing interests.

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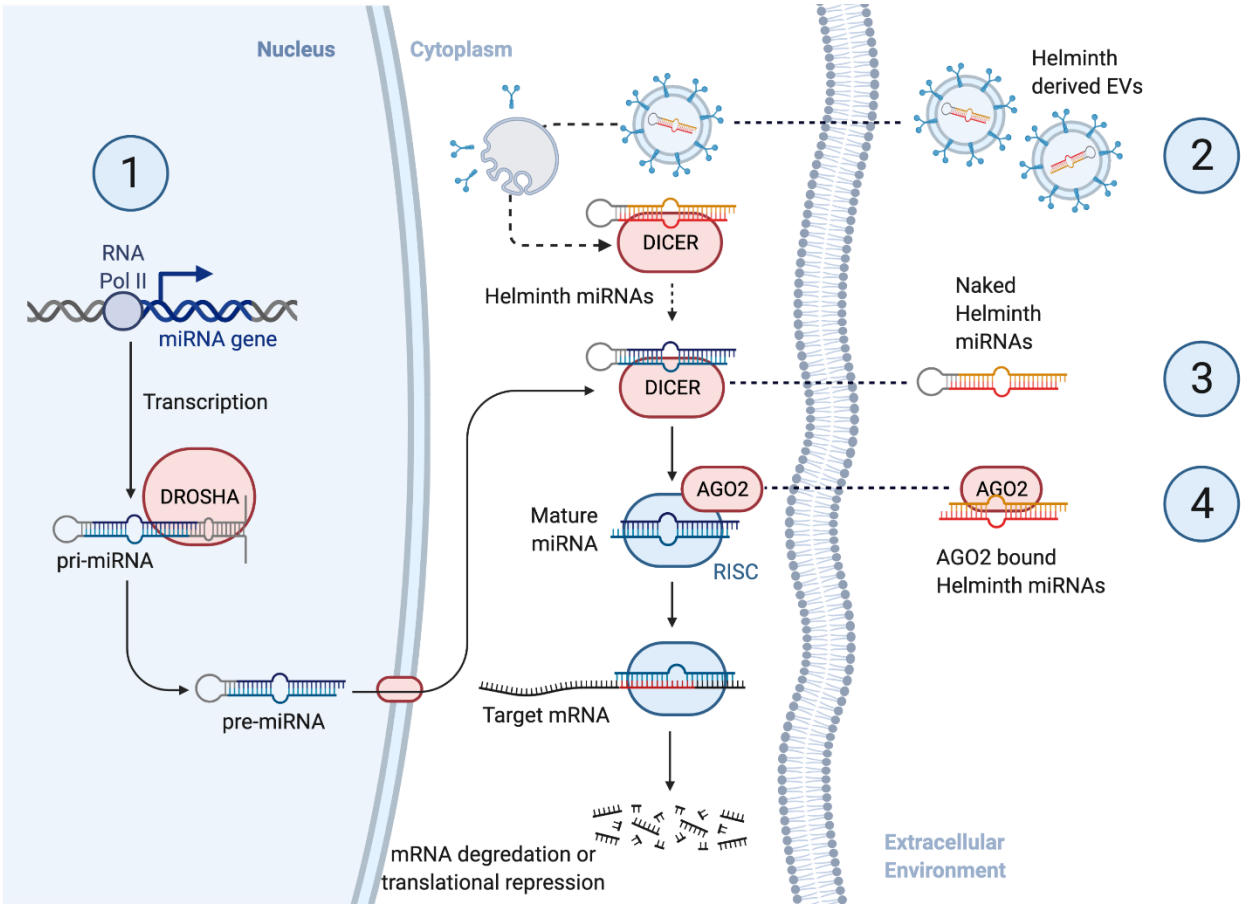

Figure 1. Propose model for the uptake of helminth miRNAs into host miRNA pathways. In mammalian cells, the canonical miRNA biogenesis pathway involves the transcription of the nascent primary miRNA transcript. This is followed by an orchestrated process of enzymatic cleavage involving Drosha and Dicer to generate the mature miRNA strand which is then loaded onto AGO2 for functionalisation (Pathway 1). Helminth miRNAs can entry this pathway using EVs bearing helminths miRNAs (Pathway 2) which are taken up by host cells. Another alternative route is the uptake of naked or AGO2 bound helminth miRNAs released into the extracellular environment (Pathway 3 and 4). By entering the host miRNA regulatory network, these helminths miRNAs have the potential to regulate host genes and alter pathways.