New roles for microRNAs in cross-species communication

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Communication between cells ensures coordinated behavior. In prokaryotes, this signaling is typically referred to as quorum sensing, whereas in eukaryotic cells, communication occurs through hormones. In recent years, reports have shown that small noncoding RNAs, called microRNAs (miRNAs), can be transmitted from one species to another, inducing signal interference in distant species, even in a cross-kingdom manner. This new mode of cross-species communication might mediate symbiotic and pathogenic relationships between various organisms (e.g., microorganisms and their hosts). Here, we discuss several recent studies concerning miRNA-mediated cross-species gene regulation.

Introduction

Organisms do not exist in isolation; instead, most organisms communicate through interconnected ecosystems. The research field of cross-species communication has received significant attention in recent years. This concept is defined as the communication between different species of animals, plants, fungi and bacteria through an array of hormones or hormone-like chemicals. This field evolved from the initial concept in the early 1980s that different cell types can communicate with each other through secreted hormones.[1] Subsequently, this field expanded with the realizations that bacterial signals modulate mammalian and plant cell signal transduction and that host hormones also cross-signal with quorum-sensing molecules to modulate bacterial gene expression.[2-4]

Recently, scientists have discovered an entirely new level of gene regulation mediated through small molecules called microRNAs (miRNAs). miRNAs are a class of single-stranded noncoding RNAs of approximately 22 nucleotides in length that play an important role in post-transcriptional gene regulation.[5,6] It is now accepted that miRNAs play crucial regulatory roles in numerous biological processes, including cell proliferation, differentiation, development, apoptosis and metabolism.[7] Strikingly, recent findings have suggested that miRNAs not only execute functions within the original cells but can also be transmitted from one species to another, facilitating cross-talk, communication or signal interference between different species, even in a cross-kingdom manner. The identification of this novel mechanism has greatly enhanced our understanding of molecular signaling between species. Here, we discuss several recent studies concerning miRNA-mediated cross-species gene regulation.

Plant miRNAs regulate gene expression in mammals. Our recent studies utilizing oxidized deep sequencing and quantitative RT-PCR revealed that plant miRNAs bearing 2'-O-methylated 3' ends could stably exist in the sera and tissues of humans and herbivorous animals at concentrations similar to some endogenous mammalian miRNAs.[8] Furthermore, using a murine model, we demonstrated that plant miRNAs accumulate in the serum and tissues as a result of food intake and that exogenous mature plant miRNAs in food pass through the mouse gastrointestinal tract.[8] Moreover, we showed that, in mammals, low-density lipoprotein receptor adaptor protein 1 (LDLRAP1) is a target of MIR168a, which is one of the plant miRNAs present at relatively high levels in human serum, and MIR168a directly binds to the coding sequence of LDLRAP1 in human liver cells and influences the uptake of LDL from the blood.[8] This finding provides evidence that food-derived exogenous plant miRNAs enter into the circulation and organs of mammals, where they serve functions similar to endogenous miRNAs, simultaneously regulating multiple target genes and biological processes.

However, the uptake of plant-derived miRNAs into the body through food sources and eventual absorption into the target organs remains challenging. Once inside the mammalian gastrointestinal tract, exogenous miRNAs encounter RNases, phagocytosis and a low-pH environment, unfavorable conditions that might require the miRNAs to adopt stable structures for protection against degradation prior to reaching the recipient cells. However, the mechanism underlying the absorption of exogenous plant miRNAs into intestinal epithelial cells remains unclear. It is also unknown how plant miRNAs are eventually delivered to the cells of target organs, such as the liver in mammals, after uptake. Thus, it is essential to characterize the mechanisms underlying the absorption and processing of exogenous plant miRNAs in mammalian cells.

Human miRNAs affect malaria parasite biology and survival. Recently, LaMonte et al. reported that human miRNAs could be translocated into the malaria parasite *Plasmodium falciparum*, where they are incorporated covalently into *P. falciparum*

mRNAs to repress mRNA translation, leading to a modest but significant reduction in the growth of *P. falciparum*.[9] Specifically, these authors detected the presence of 100 human miRNAs within the parasites using multiplex real-time PCR assays. Interestingly, some human miRNAs, which are abundant in HbAS and HbSS erythrocytes (e.g., miR-451), were significantly enriched in the parasite.[9] Using northern blot analyses, these authors verified that the intra-parasitic miRNAs were fully intact mature human miRNAs and ruled out the possibility that the parasitic miRNAs are derived from host erythrocyte contamination.[9] To validate the miRNA uptake profile across the intraerythrocytic developmental cycle (IDC), LaMonte et al. examined three miRNAs and observed that let-7i was maximally expressed after 16 h of parasite growth, whereas the expression of miR-451 peaked after 32 h.[9] These differences strongly suggest that the uptake of miRNA might be an active, dynamic process. Moreover, miR-451 was localized within the parasitophorous vacuolar membrane (PVM).[9] Subsequently, using Illumina deep sequencing, 5' RACE and ribonuclease protection assays, these authors confirmed that the human miRNAs transferred into the parasite (e.g., miR-451) formed chimeric fusions with *P. falciparum* mRNA and, via impaired ribosomal loading, resulted in translational inhibition, which eventually affected the parasite biology and survival.[9] Thus, sickle cell erythrocytes exhibit cell-intrinsic resistance to malaria in part through an atypical miRNA activity, which may represent a unique host defense strategy against complex eukaryotic pathogens.

This study was the first to show that human miRNAs regulate protozoan gene expression and provides an explanation for the parasite resistance typically observed in the red blood cell disorder β-thalassemia. However, this study also raises some questions. First, it is currently unknown how miRNAs are incorporated into malaria parasites. Plasmodium parasites are contained within a parasitophorous vacuolar membrane; the miRNA would have to cross through both the red blood cell and parasite plasma membranes. Thus, it will be of great interest to elucidate the mechanism of miRNA translocation, and the direct visualization of miR-451 activity in the parasitophorous vacuolar membrane should be reconciled. Second, Plasmodium parasites do not contain Dicer or Argonaute homologs that comprise the RNA-induced silencing complex (RISC). RNA interference, including the traditional function of miRNAs, is not observed in these organisms. Thus, it is not surprising that translocated miRNAs are covalently linked to certain Plasmodium mRNAs and form chimeric RNAs, independent of the conventional role of miRNAs in post-transcriptional gene regulation. Covalent linkage requires a trans-splicing event; however, among unicellular eukaryotes, trans-splicing has only been observed in kinetoplastid organisms, whereby a leader sequence is spliced onto the 5' end of all RNA transcripts during processing and maturation.[10] In *P. falciparum*, miRNA tagging occurs for a minority of transcripts, including regulatory subunit of cAMP-dependent protein kinase (PKA-R), phosphoethanolamine N-methyltransferase (PEAMT) and 28S and 18S rRNAs. The mechanism that determines the specific enrichment of certain miRNAs or the incorporation of these miRNAs into specific parasite mRNAs is

unknown, and whether Argonaute proteins are involved in these processes has not been determined.

The regulation of viral and host gene expression occurs through miRNAs. Another example of miRNA-mediated cross-species communication comes from virus-host interactions. Numerous studies have demonstrated that viruses hijack the host cell miRNA machinery to generate their own miRNAs.[11-13] Although the functions of most viral miRNAs remain unknown, current evidence indicates that viruses use miRNAs to manipulate both viral and host gene expression.[14-21] In general, viral miRNAs target cellular genes involved in cell proliferation, survival, stress responses or antiviral defense pathways, as prolonging cell survival and evading immune recognition are at least two mechanisms by which viral miRNAs facilitate the virus life cycle.[16-21] Thus, viruses take advantage of the host cell miRNA machinery and establish a cellular environment conducive to viral replication. More interestingly, a handful of viral miRNAs exhibit homology to human oncogenic miRNAs. For example, Kaposi's sarcoma-associated herpesvirus (KSHV)-encoded miR-K12-11 is an ortholog of miR-155.[22,23] One shared cellular target of miR-K12-11 and miR-155 is BACH-1 (Btb and CNC homolog 1), a transcriptional repressor involved in the oxidative stress response and cell cycle regulation.[22,23] Given the known etiological role of miR-155 in B-cell transformation, it has been suggested that miR-K12-11 might contribute to the induction of KSHV-positive B-cell tumors in infected patients.[22,23] Hence, viruses might utilize the molecular mimicry of miRNA genes to regulate host cellular environments in the same manner as the homologous cytokines, growth factors and immunomodulatory genes commonly found in virus genomes.

There are other mechanisms by which host miRNAs influence the life cycles of viruses. It has been shown that miR-24 and miR-93 target viral large protein (L protein) and phosphoprotein (P protein) genes in mice infected with vesicular stomatitis virus (VSV).[24] Similarly, a cluster of cellular miRNAs, including miR-28, miR-125b, miR-150, miR-223 and miR-382, target the 3' ends of HIV-1 mRNAs in cultivated resting CD4$^+$ T cells to maintain HIV-1 latency.[25] Moreover, it has been reported that miR-32 effectively restricts the accumulation of retrovirus primate foamy virus type 1 (PFV-1) in human cells in a direct, sequence-specific manner.[26] Therefore, through the fortuitous recognition of foreign nucleic acids, cellular miRNAs exert direct antiviral effects in addition to their conventional post-transcriptionally regulatory functions. Perhaps the role of miR-122 in controlling hepatitis C virus (HCV) infection is the most interesting example of the control of viruses through host miRNAs. miR-122 stimulates HCV replication through unique interactions at two binding sites in the 5' UTR of the HCV genome.[27] In this context, miR-122 stabilizes viral RNA and reduces its decay rather than inducing viral RNA degradation, thereby expanding the knowledge of how miRNAs modulate gene expression in multiple ways.[27]

Taken together, these data show that while viral miRNAs profoundly impact host gene expression to augment viral replication, host miRNAs directly interact with viruses. These findings suggest that miRNAs play an important role in the intricate virus-host

interaction network, providing new insights into the diagnosis and control of viral diseases. However, compared with miRNAs transmitted from plants to mammals or from humans to plasmodia, there are major differences for miRNAs of viral origin. In the strictest sense, viruses are typically not considered to be individual organisms or living entities. Thus, virus-encoded miRNAs rely on the host machinery for production, whereas plant or human miRNAs are synthesized and subsequently transmitted to other organisms. Therefore, the miRNA-mediated cross-species communication between viruses and hosts is somewhat different from that observed in other systems.
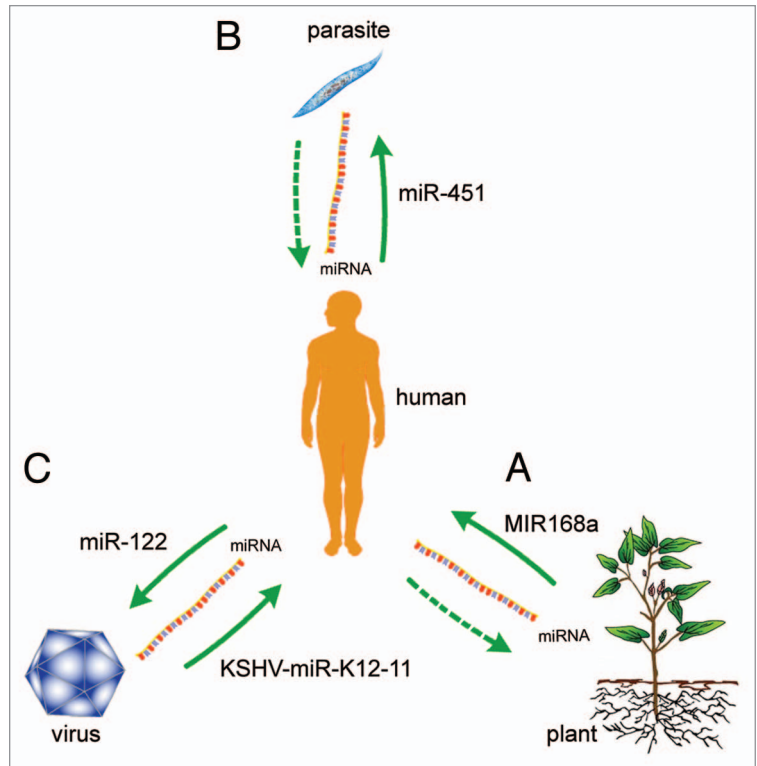
Conclusion

Once considered unstable molecules, miRNAs have recently been shown to transfer horizontally between species, suggesting a novel role for these molecules in inter-species communication. Thus, the miRNAs endogenous to one species might influence the biology of other distantly related species (**Fig. 1**).

It is not surprising that viruses and hosts regulate eachothers' gene expression patterns through miRNAs. However, for each others' species, this regulation is more complicated. Previous studies have shown that the malaria parasite does not encode miRNAs,[28] yet these pathogens might encode other small non-coding RNAs. We propose that Plasmodium regulates gene expression in host cells through small non-coding RNAs. Furthermore, we have shown that animals absorb plant miRNAs from plant foods, thereby affecting their own gene expression. However, it is unknown whether plants absorb animal miRNAs from animal waste or the animal ingredients in organic fertilizers to affect plant gene expression. Thus, interspecies miRNA interactions will be a new and exciting field of study, and much remains to be learned about the mechanisms and roles of miRNAs in both normal physiological and pathological contexts before we can fully understand exogenous miRNA-mediated cross-species communication.

Figure 1. Cross-species regulation of gene expression through miRNAs. (**A**) Plant miRNAs (e.g., MIR168a) regulate gene expression in mammals.[8] (**B**) Human miRNAs (e.g., miR-451) affect malaria parasite biology and survival.[9] (**C**) Viral miRNAs (e.g., KSHV-miR-K12-11) regulate host gene expression,[16-23] and host miRNAs (e.g., miR-122) regulate viral gene expression.[24-27]

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