Combined agonist–antagonist genome-wide functional screening identifies broadly active antiviral microRNAs

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Although the functional parameters of microRNAs (miRNAs) have been explored in some depth, the roles of these molecules in viral infections remain elusive. Here we report a general method for global analysis of miRNA function that compares the significance of both overexpressing and inhibiting each mouse miRNA on the growth properties of different viruses. Our comparative analysis of representatives of all three herpesvirus subfamilies identified host miRNAs with broad anti- and proviral properties which extend to a single-stranded RNA virus. Specifically, we demonstrate the broad antiviral capacity of miR-199a-3p and illustrate that this individual host-encoded miRNA regulates multiple pathways required and/or activated by viruses, including PI3K/AKT and ERK/MAPK signaling, oxidative stress signaling, and prostaglandin synthesis. Global miRNA expression analysis further demonstrated that the miR-199a/miR-214 cluster is down-regulated in both murine and human cytomegalovirus infection and manifests similar antiviral properties in mouse and human cells. Overall, we report a general strategy for examining the contributions of individual host miRNAs in viral infection and provide evidence that these molecules confer broad inhibitory potential against multiple viruses.

RNAi | herpesvirus | RNA virus | RNA processing | phosphatidylinositol-3-kinase-Akt signalling

Since the discovery of the first microRNA (miRNA) in *Caenorhabditis elegans*, research in diverse organisms has illuminated the role of this class of small RNA in a wide range of cellular processes (reviewed in ref. 1). MicroRNAs modulate the expression of specific genes by guiding the RNA-induced silencing complex (RISC) to complementary sites within messenger RNAs (mRNAs) (2). This generally serves to down-regulate target genes at specific times, in concert with other regulatory mechanisms in the cell (reviewed in ref. 3). Functional analysis of individual miRNAs suggests diversity in the timing and mechanisms by which they regulate cellular events. For example, some miRNAs promote cellular proliferation, whereas others promote apoptosis (depending on which genes are targeted). Consequently, the expression level of a given miRNA within a cell is expected to be under tight regulatory control, and mechanisms for achieving this control continue to emerge (reviewed in ref. 4).

Viruses require host-cell processes for their survival and have evolved mechanisms for modifying cellular conditions toward an environment conducive to replication while evading recognition and destruction by the host. Herpesviruses are one of the oldest and most successful viral families in this regard. They have coevolved with their hosts for hundreds of millions of years and infect nearly all vertebrate species studied and at least one invertebrate (5). Human cytomegalovirus (HCMV), a member of the β-herpesvirus subfamily that infects a large portion of the world's population (50–90%), is a leading cause of congenital infection (~1% of live births) and a major cause of morbidity in immunocompromised patients. Although mouse and human cytomegaloviruses have diverged over ~80 million years, the pathophysiology of murine CMV (MCMV) in mice is similar to that of HCMV in humans, and the lytic infections result in activation and manipulation of common host-cell signaling cascades (6).

Because miRNAs regulate many aspects of cellular physiology, their expression levels could impact the infection process. It might be expected, therefore, that host miRNA expression is subject to regulation upon infection, by either viral or host factors. Indeed, we and others have previously identified host miRNAs that are down-regulated upon infection by cytomegaloviruses (in some cases within 4 h postinfection) and have demonstrated that these miRNAs exert antiviral properties when overexpressed (7, 8). However, to date, there has been no overlap between results reported with MCMV and HCMV, nor has there been any context with which to interpret the significance of the effect of overexpressing a given miRNA in relation to any other miRNA in the cell. Similarly, various groups have identified mammalian miRNAs that are regulated or implicated in diverse viral infections, but the majority of these studies are generally founded on expression profiling or miRNA target predictions, (reviewed in ref. 9; ref. 10). There is relatively little investigation to date on the functional impact of miRNAs in different infections.

We postulate that specific subsets of host miRNAs are important in controlling the infection process and might be subject to regulation by host and/or viral factors. Although the kinetic parameters of miRNA action are not well-known (e.g., how quickly and reversibly they can modulate a gene or pathway), we expect that viruses with slower replication kinetics (>24 h) might be particularly sensitive to (and exploitive of) changes in host miRNA expression levels. Here we report a combined agonist–antagonist miRNA screening approach that is designed to obtain functional information about mouse miRNAs that impact the lytic phase of herpesviral infections. We further test the breadth of observed antiviral miRNA properties in human cells and against an evolutionarily unrelated RNA virus. Our functional and expression analyses demonstrate that host miRNAs are a tunable and important component of herpesviral infection and provide evidence that these molecules have broad antiviral properties in mouse and human cells against both DNA and RNA viruses.

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Conflict of interest statement: A.V. is, as of the publication date, employed, with fixed salaries, by Thermo Fisher Scientific, which offers for sale libraries of miRNA mimics and inhibitors.

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