JOURNAL OF INTERFERON & CYTOKINE RESEARCH
Volume 30, Number 11, 2010
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DOI: 10.1089/jir.2010.0080

Interferons and MicroRNAs

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Plants rely heavily on an adaptive RNA degradation system mediated by an RNA interference mechanism to combat viral infection, whereas mammals fight infection with specific antibodies and lymphocytes that are adapted to specific viral antigens, and also employ nonadaptive defenses, such as production of interferons (IFNs) that block viral replication and stimulate the host immune response. Therefore, the IFN system represents an integral part of the mammalian antiviral innate immunity, and it is not surprising to find that cellular, IFN-regulated microRNAs contribute to this antiviral defense. In contrast, virus-encoded microRNAs target host cell factors that are either required for the induction of IFNs after pathogen recognition, or are involved in the cellular responses to these pleiotropic cytokines.

Introduction

When the first microRNA (miR), lin-4, was discovered in 1993 due to its repressive properties on the translation of the lin-14 mRNA, it was impossible to foresee the enormous impact this finding would have on all aspects of cell biology. Yet, it has become rapidly clear that miRs are crucial posttranscriptional regulators of gene expression by decreasing the abundance or translational efficiency of mRNAs (Maroney and others 2006; Nilsen 2007). Bioinformatics analysis of putative miR targets suggests that up to 30% of all human genes might be regulated by miRs. While the role of miRs in cell fate decisions associated with cell proliferation, differentiation, and apoptosis was recognized early on, the importance of these noncoding small RNAs on immune system development and on immune responses has only more recently become evident.

In addition to facilitating cell fate decisions of immune cells (Sonkoly and others 2008), miRs have more recently been shown to play an important role in host–virus interactions. Evidence exists that cellular miRs not only alter immune cell development and function, but are also able to directly affect viral replication. Conversely, virus-encoded miRs shape the host–virus interactions and regulate the viral life cycle, thereby attesting to the fact that both mammalian cells and viruses employ miRs, the first as part of their innate immune response, and the latter to avert the same (Gottwein and Cullen 2008; Pedersen and David 2008). As the interferon (IFN) system represents an integral part of the mammalian innate immunity, it is not surprising to find that cellular, IFN-regulated miRs contribute to this antiviral defense, whereas virus-encoded miRs target host cell factors that are either required for the induction of IFNs after pathogen recognition, or are involved in the cellular responses to these pleiotropic cytokines. This summary aims to provide a brief overview of the connections between cellular and viral miRs and the IFN system.

Interferon Regulation of Cellular miRs

Mammals fight infection with specific antibodies and lymphocytes that are adapted to specific viral antigens, but they also employ nonadaptive defenses, such as production of IFNs that block viral replication and stimulate the host immune response. However, none of these defense mechanisms have been found in plants. Instead, plants appear to rely heavily on an adaptive RNA degradation system mediated by an RNA interference mechanism (Ding and others 2004; Roth and others 2004; Zamore 2004; Wang and Metzlaff 2005). Indeed, inhibition of RNA interference (RNAi) in plants increases their susceptibility to many plant viruses. These results naturally raise the following question: Do mammalian cells actually mount a protective RNAi response after viral infection? Indeed, not too long ago a review article stated that "[I]t seems reasonable to propose that the extremely potent IFN system has displaced RNAi as the key defense against virus infection in mammalian cells." Even though artificially induced RNAi responses could confer protection against a wide variety of pathogenic viruses in mammalian cells, there was no evidence that inhibition of the RNAi response could enhance virus replication in mammalian cells. Leceillier and others (2005) then reported that in cells expressing a suppressor of RNA silencing, accumulation of the retrovirus, primate foamy virus type 1, was strongly enhanced, indicating involvement of siRNAs or miRNAs in the control of virus replication. Further, another report indicated that the human immunodeficiency virus (HIV)-1 Tat proteins have evolved to function as a suppressor

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of RNA silencing to combat cellular defenses (Bennasser and others 2005).

Dicer is a key enzyme in the generation of miRs, and its absence precludes the formation of mature miRs. The abrogation of Dicer expression in mice results in hypersensitivity to vesicular stomatitus virus (VSV) infection (Otsuka and others 2007), and knockdown studies in cell culture implicated Dicer in the protection against influenza A virus (Matskevich and Moelling 2007). It is therefore surprising that double-stranded RNA and type I IFNs were reported to inhibit Dicer expression (Wiesen and Tomasi 2009). Nonetheless, this broad ablation of miR expression due to the absence of Dicer illustrates the importance of miRs in the mammalian innate immune response in general, and more information is now becoming available on the specific roles of distinct miRs in the IFN-induced antiviral host response.

Inhibition of viral replication through the modulation of cellular miR expression might present in 2 ways: either IFNs induce antiviral miRs, or they inhibit expression of cellular miRs that support the viral life cycle. Indeed, several viruses have now been shown to take advantage of host cell miRs to enhance their replication, and the latter scenario is effectively demonstrated by the strong positive effect of the liver-specific miRNA miR-122 on the replication of hepatitis C virus (HCV) (Jopling and others 2005). There, abrogation of miR-122, which targets the 5′ noncoding region of the HCV genome, resulted in a marked loss of autonomously replicating HCV viral mRNAs. The mechanism underlying the effect is at this point not well understood, but miR-122 is thought to primarily act by enhancing viral RNA replication rather than viral mRNA translation or stability. Regardless, we and others observed that IFNα/β treatment of the human hepatocyte cell line Huh7 leads to a temporary attenuation of miR-122 expression by 20%–40% (Pedersen and others 2007; Sarasin-Filipowicz and others 2009; Gong and others 2010). However, at this point it does not seem likely that this phenomenon contributes appreciably to the efficacy of type I IFNs in the treatment of HCV infected individuals, as no correlation between intrahepatic miR-122 and HCV load or IFN responsiveness was evident in liver biopsies from HCV-infected patients (Sarasin-Filipowicz and others 2009).

In addition to the downregulation of miR-122 in Huh7 cells, our lab had also identified cellular miRs whose expression increased in response to IFNβ stimulation, and ectopic expression of the corresponding synthetic miR-mimics attenuated HCV replication *in vitro* (Pedersen and others 2007). Several of these IFN-induced miRs (miR-196, miR-296, miR-351, miR-431, and miR-448) displayed seed sequence matches within the HCV genome and mutation of the predicted target sites of miR-196 and miR-448 in the HCV genome obliterated the inhibitory effect of these miRs on HCV replication (Pedersen and others 2007). Similarly, Murakami and others (2009) described the inhibition of the HCV genome replication by miR-199a.

The notion that antiviral cellular miRs not only regulate host cell gene expression, but also target foreign nucleic acids is strongly supported by several reports involving different viruses. A study by Nathans and others (2009) demonstrated a direct interaction of miR-29a with the HIV genomic RNA, and consequent inhibition of HIV replication. miR-29a is highly expressed in HIV-infected T cells, and appears IFNα/β-inducible in uninfected cells (our unpublished observation). Several additional cellular miRs have been proposed to directly interact with HIV genomic RNA sequences (Hariharan and others 2005). Similarly, miR-32 restricts the replication of primate foamy virus type 1 (Lecellier and others 2005). Two other human miRs, miR-507 and miR-136, have been proposed to target binding sites in the polymerase and hemagglutinin genes of influenza A virus (Scaria and others 2006), and the spectrum of cellular miRs restricting influenza A virus replication was recently expanded to include miR-323, miR-491, and miR-654 (Song and others 2010).

Loss of miR-24 and miR-93, which inhibit expression of Vesicular Stomatitis Virus–encoded proteins, promotes the replication of VSV, and mutant strains lacking the target sites for miR-24 and miR-93 replicate more efficiently in wild-type but not in Dicer$^{-/-}$ animals (Otsuka and others 2007). Even though there is little doubt about the antiviral activity of these miRs, it still needs to be established whether they are subject to regulation by the type I IFN system during an immune response. Conversely, the induction of miR520b by IFNγ and the resulting downregulation of the NKG2D ligand MHC class I–related chain A has been described, but the extent to which this process modulates antiviral immunity needs to be determined (Yadav and others 2009).

An obvious question that emerges is why viruses subject to repression by cellular miRs have not mutated to escape this inhibition. One possibility is that the sequences targeted by the antiviral miRs are crucial elements of the viral genomes or transcripts. However, the possibility that the attenuation of viral replication through cellular miRs is actually beneficial to the virus has to be also considered. Self-limiting virulence might allow the invading virus to prevent an all-out immune response, and could be a contributing factor in the establishment of viral latency and chronic infection. This notion finds support in the fact that virus-derived miRs can act in an auto-inhibitory manner (Omoto and others 2004).

The IFN System as a Target for (Viral) miRs

It is not surprising to find that viruses have evolved to utilize miRs to their advantage, as they require comparatively little coding space in the viral genome, while at the same time remaining undetected due to their lack of antigenicity. Indeed, several viral miRs have been implicated in immune evasion strategies; for instance, it has been reported that the herpes simplex virus-1 latency-associated transcript encodes an miR precursor whose mature product apparently targets the transforming growth factor β (TGFβ)/SMAD pathway (Gupta and others 2006; Umbach and others 2008). Kaposi's sarcoma-associated herpes virus (KSHV) was reported to harbor at least 12 miRs (Cai and others 2005; Cai and Cullen 2006) cooperatively targeting osteopontin and thrombospondin-1 (THBS1). THBS1 activates TGFβ, and its suppression by KSHV-encoded miRs might account for the reduced TGFβ activity correlated with KSHV pathogenesis (Samols and others 2007). The MHC class I–related chain B transcript, which encodes a ligand for the natural killer (NK) cell receptor NKG2D, was recently identified as the target of an miR encoded in the UL112 region of human cytomegalovirus (Stern-Ginossar and others 2007). Several miRs derived from the HIV-1 genome were similarly shown to target both cellular and viral transcripts during HIV-1 infection (Omoto and others 2004; Pfeffer and others 2004; Bennasser and others 2005; Ouellet and others 2008).

Parameswaran and others (2010) conducted what is probably the most comprehensive search for viral miRs to date, and identified virus-derived miRs in varying abundance in cells infected with Hepatitis C, Polio, Dengue, Vesicular Stomatitis, and West Nile viruses.

Considering the vital role of the IFN system, it is likely that some of the viral miRs might either target pattern recognition receptors and signaling components involved in the IFN induction after pathogen recognition, or alter expression of proteins involved in the IFN response such as the Janus kinase (Jak)/signal transducer and activator of transcription (STAT) pathway. No published evidence experimentally confirming attenuation of the IFN system by viral miRs exists to date; however, several cellular miRs that are induced during viral infection are negatively modulating the IFN pathway.

A recent study illustrated that 2 cellular miRs, miR-221 and miR-222, are functioning to attenuate both expression and phosphorylation of STAT1 and STAT2 in response to IFNα (Zhang and others 2010). In addition, miR-26a, miR-34a, miR-145, and let-7b appear to directly regulate IFNβ expression (Witwer and others 2010). It is feasible that these miRs, which intriguingly are also IFNβ-inducible and thus likely part of a negative feedback loop, are exploited by viruses to restrain the IFN response during infection.

miR-146 and miR-155 are also crucially involved in innate immunity by regulating the acute inflammatory response after pathogen recognition by Toll-like receptors (TLRs) on monocytes or macrophages (Taganov and others 2006; O'Connell and others 2007). Inducible expression of miR-155 was observed during both bacterial and viral infections, as well as after exposure of cells to proinflammatory cytokines such as IFNβ, IFNγ, or tumor necrosis factor α (TNFα). In contrast, miR-146 increases were mostly restricted to induction by bacterially derived ligands or IL-1 and TNFα (Taganov and others 2006; O'Connell and others 2007). Both miRs appear to function as components of negative feedback loops attenuating TLR signaling pathways, whereby Fas-associated protein with death domain (FADD), receptor-interacting protein (RIP), and inhibitor of nuclear factor kappa-light-chain-enhancer of activated B cells kinase epsilon (IKKε) are suppressed by miR-155 (Tili and others 2007), and miR-146 limits IRAK1 and TRAF6 expression (Taganov and others 2006). Importantly, miR-146a directly attenuated transcriptional induction via the IFNα/β receptor, presumably by limiting expression of STAT1 (Tang and others 2009).

Another IFN-induced innate immune response component that lends itself to speculation in the context of miR function is the potent upregulation of the RNA editing enzyme Adenosine deaminase acting on RNA 1 (ADAR1). ADAR1 is one of the most highly IFNα/β-inducible proteins, and is capable of deaminating not only cellular and viral mRNA, but also miRs (Yang and others 2006; Das and Carmichael 2007). Editing of mir-142, the precursor of miR-142, suppresses its processing by Drosha, and leads to the degradation of the pri-mir-142 by the Tudor-SN complex (Yang and others 2006). The question therefore arises: Is the IFNα/β-induced ADAR1 interfering with the function of viral miRs by editing their precursors?

Conclusion

Whether host cells derived as part of the IFN-inducible innate immune system, or encoded by invading viruses to circumvent host responses, miRs as posttranscriptional regulators of the immune response have added yet another weapon to this ongoing arms race. The constantly increasing number of virally encoded miRs further obscures an already complex system of posttranscriptional gene regulation facilitated by the combinatorial and sometimes redundant functions of cellular miRs during the host immune response.

Author Disclosure Statement

No competing financial interests exist.

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Received 8 July 2010/Accepted 8 July 2010