CHAPTER 4

Micromanagers of Immune Cell Fate and Function

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Contents

Abstract

MicroRNAs (miRNAs) regulate cell fate during development and in response to environmental cues. Here, we review the emerging story of how miRNAs regulate immune cell development and function.

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1. INTRODUCTION

RNA interference (RNAi) is a powerful, highly specific and ubiquitous gene silencing mechanism that regulates the expression of important genes that control cell differentiation and survival (Ambros, 2004; Bartel, 2004; Dykxhoorn and Lieberman, 2005). RNAi was originally discovered in lower organisms, where it plays an important role in regulating development and cell differentiation and protecting the genome from viruses and transposable elements that can insert into and damage chromatin (Ambros, 2004; Du and Zamore, 2005; Dykxhoorn *et al.*, 2003). Seminal papers by Lee and Ambros describing a noncoding stem-loop RNA (lin-4) that regulates larval development in *Caenorhabditis elegans* (Lee *et al.*, 1993) and by Fire and Mello describing unanticipated gene silencing by small double-stranded RNAs in *C. elegans* triggered an explosion of research to understand how cells use small RNAs to regulate gene expression (Fire *et al.*, 1998). RNAi was only shown to operate in mammalian cells in 2001 (Elbashir *et al.*, 2001). Since then, this endogenous mechanism of gene silencing has become a powerful research tool for manipulating gene expression to understand the function of individual genes or to perform unbiased genetic screens in mammalian systems. In addition, researchers in academia and industry are actively investigating how to harness this endogenous and ubiquitous process to manipulate gene expression to treat a diverse array of diseases, including inflammatory and autoimmune diseases and infection (de Fougerolles *et al.*, 2007).

2. miRNA BIOGENESIS

Although there are multiple types of small double-stranded RNAs in cells (Ghildiyal and Zamore, 2009; Seto *et al.*, 2007), some of which are just being discovered by deep sequencing, the dominant class of endogenous small RNAs in somatic cells is the microRNA (miRNA) (Ambros, 2004; Bushati and Cohen, 2007; Du and Zamore, 2005; Dykxhoorn *et al.*, 2003). miRNAs are transcribed as stem-loop structures, either on their own or within miRNA clusters in longer primary transcripts (pri-miRNA) that are typically encoded within intergenic regions or introns. The expression of miRNA primary transcripts appears to be regulated via enhancers and promoters much like coding genes. In fact, transcription factors and miRNAs can sometimes coregulate each other (Chang and Mendell, 2007; Petrocca *et al.*, 2008). The promoters for intergenic miRNAs, however, have mostly not yet been defined. Through a series of sequential nuclear and cytoplasmic steps, the pri-miRNAs are processed by a family of related RNase enzymes into small imperfectly paired ~22 nt double-stranded

miRNAs. pri-miRNAs are first cleaved by the RNase Drosha in the nucleus, which converts them to shorter stem-loops (pre-miRNA) that are exported to the cytoplasm by Exportin V. In the cytoplasm, the loop of the pre-miRNA is removed by the RNase Dicer to yield a small imperfectly paired double-stranded RNA of ~22 nt in length, which is handed to the RNA-induced silencing complex (RISC) (Bartel, 2004). Recent studies suggest that these processing steps may also in some cases be highly regulated (Newman *et al.*, 2008; Thomson *et al.*, 2006; Viswanathan *et al.*, 2008). The RISC localizes to perinuclear sites of RNA storage and processing (P bodies) (Bhattacharyya *et al.*, 2006). One strand of the miRNA (the antisense or guide strand) buries its 5′-end into a pocket of the RISC RNase Ago and the other strand is removed by an unclear mechanism. Removing the passenger strand activates the RISC, which can now bind mRNAs bearing partially complementary sequences to the guide strand.

3. miRNA REGULATION OF GENE EXPRESSION

In mammalian cells, miRNAs primarily regulate gene expression by blocking translation, but some targeted mRNAs may also be cleaved by the RISC or undergo accelerated degradation (Bagga *et al.*, 2005; Giraldez *et al.*, 2006; Mathonnet *et al.*, 2007, Olsen and Ambros, 1999; Pillai *et al.*, 2005, 2007; Yekta *et al.*, 2004). Therefore, reduced protein, with or without reduced mRNA, is the hallmark of miRNA targeting. The extent of gene silencing by a given miRNA on a particular protein product may be modest (Baek *et al.*, 2008; Selbach *et al.*, 2008). However, subtle changes in protein concentration can have profound physiological effects as demonstrated by the pathologies arising from haploinsufficiency. Moreover, the targeting of several components of a functional network by a single miRNA may further enhance its biological impact. Although some miRNAs may act as fine tuning rheostats to adjust gene expression subtly, there is increasing evidence that others, such as let-7 (Yu *et al.*, 2007), act as master regulators of differentiation and of the cell's response to environmental change and stress. miRNAs are estimated to regulate expression of anywhere from a third to 90% of genes (Friedman *et al.*, 2009; Lim *et al.*, 2005).

The first example of a mammalian miRNA with functional consequences, published in 2004, showed that miR-181a regulates lymphocyte development (Chen *et al.*, 2004). miRNA effects can be experimentally enhanced in cells by transfection of small double-strand miRNA mimics that resemble the Dicer cleavage product. They can also be inhibited by transfection of antisense oligonucleotides (ASO) (Krutzfeldt *et al.*, 2005). Viral vectors can also be used to express miRNAs within cells (Hannon and Conklin, 2004; Paddison *et al.*, 2004). Other approaches to antagonize

an miRNA include overexpression of transcripts with multiple miRNA recognition sites to act as a ''sponge'' and compete with endogenous mRNA binding (Ebert *et al.*, 2007).

4. miRNA TARGET GENES

Each miRNA may regulate hundreds of genes, but identifying the critical genes that are regulated by an miRNA is not straightforward and is a major stumbling block to figuring out the biological role of any individual miRNA. Nucleotides 2–9 in the active strand form a ''seed'' region; pairing of a target mRNA to this seed is an important determinant of binding, but other factors, including downstream complementarity and RNA secondary structure, are also important (Brennecke *et al.*, 2005; Hammell *et al.*, 2008; Lewis *et al.*, 2003). Although most known miRNA recognition sites in regulated gene transcripts are in the 3′UTR, recent studies suggest that sequences in the 5′UTR or coding sequences can also be important for miRNA gene regulation (Lytle *et al.*, 2007; Tay *et al.*, 2008). Current approaches to identify miRNA targets fall short of the task. The difficulty researchers face in identifying target genes, which are only partially complementary to the miRNA 22-mer active strand, is a major obstacle for understanding how miRNAs regulate cellular outcomes. The major tools that have been used are (1) bioinformatic algorithms that predict potential target genes that contain conserved complementary sequences in their 3′UTR to a seed region at the 5′-end of the miRNA active strand (Brennecke *et al.*, 2005; Doench and Sharp, 2004; Lewis *et al.*, 2003) and (2) analysis of mRNAs that are downregulated when an miRNA is overexpressed (Johnson *et al.*, 2007; Lim *et al.*, 2005). The bioinformatic approach is hampered by the fact that the existing algorithms have a high margin of error (the majority of predicted genes are not real targets and some of the key targets, such as RAS for let-7, are not predicted (Johnson *et al.*, 2007)). For many miRNAs, current algorithms predict hundreds or even thousands of potential targets, making it difficult to identify the most important targets. Gene expression array analysis does not readily distinguish direct mRNA targets from mRNAs downregulated through secondary effects and misses most target genes that are regulated by blocking translation rather than by mRNA degradation. Moreover, even when mRNA degradation occurs, changes in mRNA levels may be small (often less than twofold) and may be difficult to distinguish from background fluctuations, especially in genome-wide surveys. Combining these two approaches may work better than either approach alone, but still is not helpful in many situations. Recently, mRNA targets of miRNAs have been identified by their enrichment in coimmunoprecipitates with tagged Argonaute proteins in *Drosophila* and

human cell lines overexpressing the miRNA of interest (Beitzinger *et al.*, 2007; Easow *et al.*, 2007; Hammell *et al.*, 2008; Hendrickson *et al.*, 2008; Karginov *et al.*, 2007; Zhang *et al.*, 2007). However, these studies have not yet been shown to identify new miRNA targets. Argonaute overexpression globally increases miRNA levels, perhaps obscuring the effect of an individual overexpressed miRNA (Diederichs and Haber, 2007). Another recent approach, differential labeling with stable isotopes (SILAC), identifies proteins that are differentially expressed when an miRNA is overexpressed or antagonized (Baek *et al.*, 2008; Selbach *et al.*, 2008). This approach complements and improves upon mRNA profiling, since miRNAs have a greater effect on protein expression in mammalian cells than on mRNA. However, it does not distinguish between direct gene silencing and secondary effects. Moreover, it is a costly technology not readily available to most laboratories.

5. miRNAs REGULATE HEMATOPOIETIC DIFFERENTIATION AND IMMUNE FUNCTION

In the past 2 years, miRNAs have emerged as important regulatory elements in the control of immune cell differentiation and homeostasis and immune response (Baltimore *et al.*, 2008; Lodish *et al.*, 2008; Merkenschlager and Wilson, 2008) (Fig. 4.1). Genetic ablation, as well as ectopic or overexpression of individual miRNAs, has significant physiological consequences in the immune system, ranging from cell death and impairment of immune function to autoimmunity, lymphoproliferation, and cancer. Some pathogens, especially viruses, also encode their own miRNAs or modulate the expression of host cell miRNAs to regulate their replication or latency or to manipulate or evade host immune responses (Gottwein and Cullen, 2008).

Early evidence that miRNAs might play an important role in regulating the ordered and well-studied programs of cell differentiation that occur during immune cell development and response to antigen came from genetic manipulation of miRNA expression in mice. Infection of hematopoietic stem cells (HSC) with a lentivirus expressing a miRNA that is highly expressed in the thymus and lymphoid tissue (miR-181a) led to preferential expansion of B cells when transplanted into irradiated mice (Chen *et al.*, 2004). Conditional genetic ablation of Dicer, the RNase required to generate mature miRNAs from precursor transcripts, in immature DN3 thymocytes by use of Cre recombinase expressed from an lck promoter (lck-cre) to generate Dicer$^{fl/fl}$lck-cre mice profoundly reduced thymocyte numbers (~10-fold) and there was a virtual absence of T cells in the periphery. If Dicer was deleted somewhat later in thymocyte development in double positive (DP) thymocytes via CD4-cre then

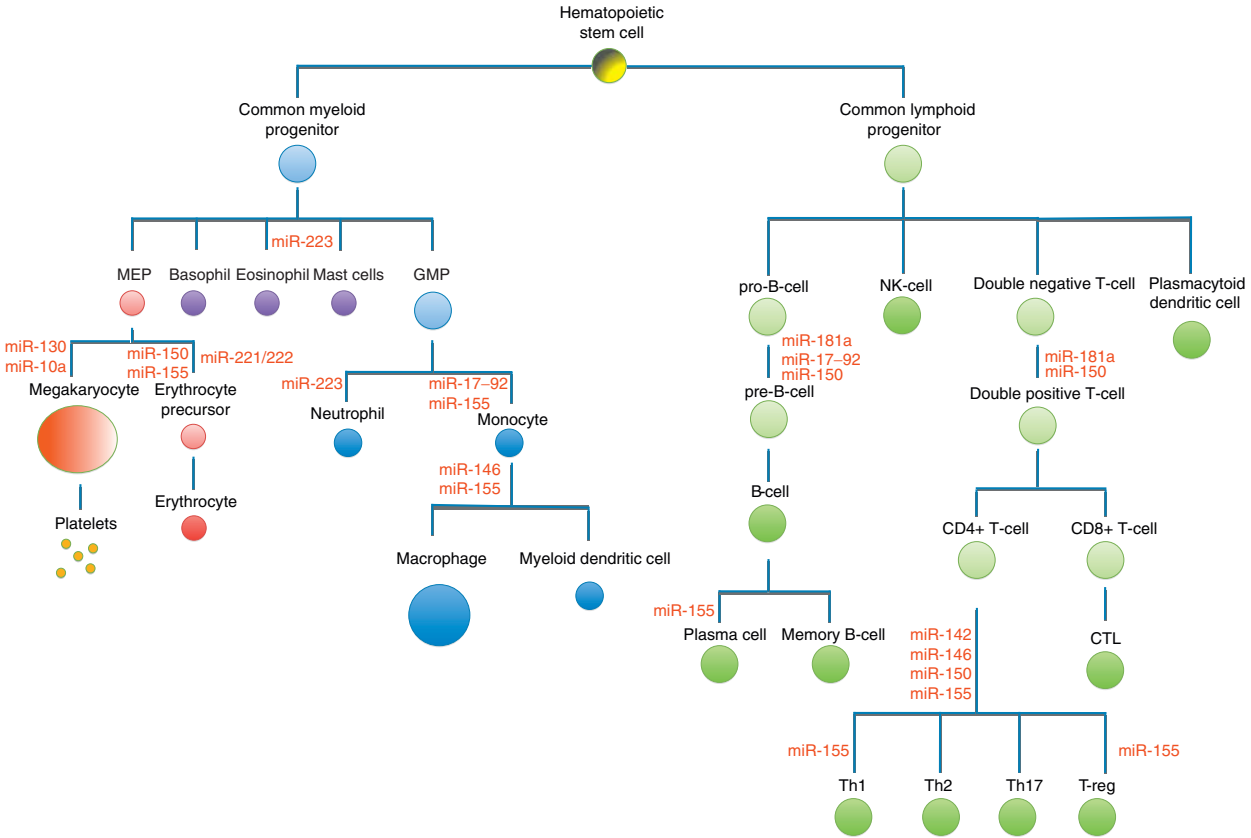

FIGURE 4.1 miRNAs implicated in hematopoiesis. Figure has been adapted from Baltimore *et al.* (2008).

peripheral T cell numbers were only moderately reduced but CD4 T cells developed aberrantly, favoring differentiation into T_H1 cells (Muljo *et al.*, 2005). There was also a substantial reduction in T_{REG}, which resulted in autoimmune colitis, splenomegaly, and lymphadenopathy. In a group of recent studies, Dicer or Drosha, another miRNA-processing enzyme, were ablated specifically in T_{REG} by use of FoxP3-cre. miRNAs were found to be essential to maintain the T_{REG} phenotype and Dicer deficient T_{REG} began to express effector molecules associated with other lineages (granzymes, IL-10, IFN-γ, IL-4) and were prone to apoptosis (Chong *et al.*, 2008; Liston *et al.*, 2008; Zhou *et al.*, 2008). The effect of Dicer depletion in this lineage was uniformly fatal, resulting in autoimmune inflammation and tissue damage that was indistinguishable from the pathology that develops in the FoxP3$^{-/-}$ *scurfy* mouse. miR-155 whose transcription is upregulated by FoxP3, plays an important role in promoting T_{REG} proliferation in nonlymphopenic settings in part by suppressing Socs1 expression and thereby enhancing IL-2 signaling (Lu *et al.*, 2009). However, other miRNAs that remain to be defined are likely important in maintaining T_{REG} function, since miR-155$^{-/-}$ mice, although they have reduced T_{REG} numbers, do not develop the severe autoimmunity of mice conditionally depleted of Dicer in FoxP3^{+} T cells. These studies suggest that miRNAs may be required to maintain lineage commitment of immune cells. The miRNAs and their regulated genes that mediate the effects of Dicer knockout on T cell development and lineage commitment are largely not known.

miRNAs are needed not only for T cell development but also for B lymphocyte development. Ablation of Dicer in the earliest stages of B cell development by use of an mb-1-cre allele leads to a nearly complete block in development at the pro-B cell stage (Koralov *et al.*, 2008). The K Rajewsky and Jacks labs were able to implicate the miR-17–92 cluster family, upregulated in pro-B cells, in this process. While mice depleted of this cluster have a defect in B cell development (Koralov *et al.*, 2008; Ventura *et al.*, 2008), mice expressing a miR-17–92 transgene develop lymphoproliferative disease and autoimmunity through a defect in B cell apoptosis (He *et al.*, 2005; Xiao *et al.*, 2008). miRNAs in these gene families prevent pre-B cell apoptosis, at least in part, by suppressing the proapoptotic bcl-2 family member Bim. The Dicer knockout B cells that do develop also have altered N additions and distinct patterns of D_H gene usage during Ig gene rearrangement, suggesting an intriguing potential role of Dicer-modified small RNAs at the Ig locus affecting gene accessibility and rearrangement (Koralov *et al.*, 2008).

Recently, a group of papers have highlighted the role of individual miRNAs in both innate and adaptive immunity. These studies are likely to be the first of many examples of a substantial role of miRNAs in regulating immune cell development and response to pathogens. Although these studies clearly implicate certain miRNAs in regulating

immunity, identification of relevant miRNA-regulated immune genes is just beginning. Most of the algorithms that predict miRNA regulated target genes rely heavily on evolutionary conservation going as far back as chickens. Since many of the features of adaptive immunity have evolved more recently, many features of miRNA regulation of immune gene expression may not be evident if conservation is used to filter examination of evolving regulatory networks. This may be especially relevant for pathogen-specific responses, because many of the key human pathogenic viruses do not infect other species.

6. miRNA REGULATION OF LYMPHOCYTE DEVELOPMENT AND FUNCTION

miR-181a acts as a rheostat to regulate the sensitivity of T cells to TCR stimulation (Li *et al.*, 2007). By downmodulating the expression of multiple inhibitory phosphatases involved in TCR signaling, miR-181a enhances phosphorylation of key signaling molecules and consequently sensitivity to antigen. Modulating miR-181a expression is even able to convert an antagonistic peptide antigen to an agonistic peptide. miR-181a is highly upregulated in DP thymocytes, relative to more mature SP cells, and also enhances their sensitivity to antigen. As a consequence, manipulating miR-181a in thymocyte cultures has a profound effect on both positive and negative selection *in vitro*. Interestingly, although miR-181c has an almost identical active strand sequence to miR-181a, only pre-miR-181a is processed in thymocyte progenitor cells (Liu *et al.*, 2008). Differences in the pre-miRNA loop sequence appear to control differential processing of these two related miRNA family members.

miR-150 and miR-155, mostly expressed in hematopoietic cells, also have profound immune effects (Landgraf *et al.*, 2007; Monticelli *et al.*, 2005). miR-150 is expressed in mature B and T cells, but not earlier in development (Xiao *et al.*, 2007). When miR-150 is expressed ectopically in B cell progenitor cells, B cell development is partially blocked. Ablation of miR-155, which is highly expressed in activated B and T lymphocytes and activated macrophages and dendritic cells, leads to profound defects in a variety of immune responses. miR-155 KO mice develop autoimmune pulmonary and enteric pathology and have impaired cellular and antibody responses to pathogens and immunization (Rodriguez *et al.*, 2007; Thai *et al.*, 2007; Vigorito *et al.*, 2007). The T cell response is skewed toward T_H2 cells and c-maf, IL-10, and IL-4 gene expression is increased. The germinal center reaction is suppressed as is production of both IgM and class-switched high affinity specific antibodies. An important gene regulated by miR-155 is AID, responsible for both somatic hypermutation and class switch recombination (Dorsett *et al.*, 2008).

7. miRNA REGULATION OF INFLAMMATORY RESPONSES

miR-155 also has a profound effect on myeloid cells and is upregulated, together with miR-132 and miR-146, during the inflammatory response to endotoxin (O'Connell *et al.*, 2008; Taganov *et al.*, 2006; Tili *et al.*, 2007). miR-155 can also be induced by Type-I IFNs. miR-155 induction by both TLRs and Type-I IFN induction is dependent on activating the JNK pathway. Transducing HSC with miR-155 also leads to preferential expansion of myeloid cells, mimicking the proinflammatory effect of LPS. Some of the likely important targets for miR-155 in the response to inflammatory stress are genes that regulate transcriptional activation in myeloid cells (PU.1, Cepbb), cytokine receptors (Csfr1), and the stress response transcription factor HIF1α.

miR-146, another important miRNA family upregulated in response to endotoxin and IL-1β in monocytes and other cells, including pulmonary epithelial cells, in response to NF-κB signaling, targets downstream genes involved in cytokine and TLR signaling, including the TNF receptor-associated protein (TRAF6) and IL-1 receptor-associated kinase (IRAK1) (Perry *et al.*, 2008; Taganov *et al.*, 2006). It may also regulate expression of the chemokine receptor CXCR4 (Labbaye *et al.*, 2008). Therefore, it serves to dampen the proinflammatory response to TLR signaling. miR-146a expression is greatly increased in macrophages and some lymphocytes of synovial tissues of rheumatoid arthritis patients (Nakasa *et al.*, 2008).

8. VIRUSES HARNESS THE miRNA MACHINERY

When pathogens invade host cells, they can take advantage of the endogenous miRNA machinery to advance their own replication or modulate host immune responses to their own benefit. (A detailed description of miRNA regulation of viral infection is beyond the scope of this review; for a comprehensive review see Gottwein and Cullen (2008).) This has been studied for viruses, which can express their own miRNAs (some of which mimic host miRNAs) or modulate the expression of host miRNAs (Fig. 4.2). Viral miRNAs have been described for a variety of DNA viruses, including herpesviruses, polyomaviruses, and adenoviruses. Like eukaryotic small RNAs, these molecules are transcribed mostly from Pol II promoters, processed through the host RNAi machinery and incorporated into the host RISC. Although some studies have suggested that RNA viruses, including HIV, encode for miRNAs (Bennasser *et al.*, 2004; Omoto *et al.*, 2004), this is controversial. Studies, which identified viral miRNAs in DNA virus-infected cells, failed to detect viral small RNAs by deep sequencing small RNAs from cells infected with HIV-1

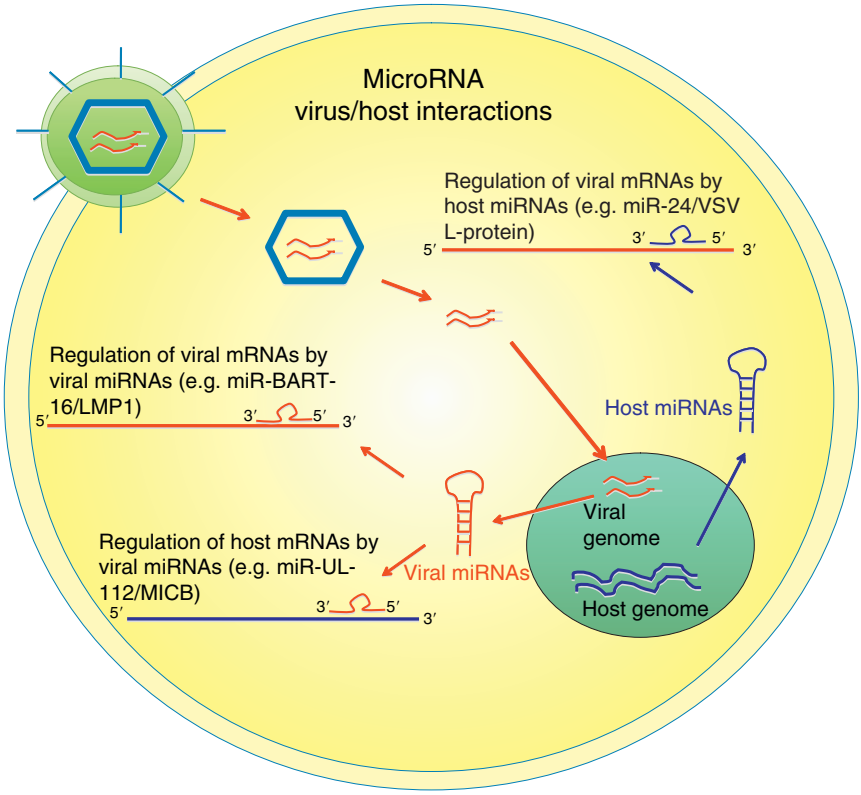

FIGURE 4.2 Both viral and host encoded miRNAs regulate gene expression to control viral infection.

or HTLV-1 (Pfeffer *et al.*, 2005). Both the Cullen laboratory (Lin and Cullen, 2007) and our unpublished data do not suggest that HIV encodes its own miRNAs.

Viral miRNAs have been most carefully studied for herpesviruses, especially those that cause human disease. Clustered miRNA polycistrons, expressed during Kaposi's sarcoma-associated herpesvirus (KSHV) and Epstein–Barr virus (EBV) latent phases and encoded in relatively conserved regions of the viral genome, regulate latency by suppressing opposite strand transcripts. One example is EBV miR-Bart2, which regulates the antisense BALF5 mRNA (Barth *et al.*, 2008). During latency, miRNAs may keep protein levels of viral genes to a minimum, facilitating evasion of immune surveillance (Huang *et al.*, 2007). Other herpesvirus miRNAs, identified in cytomegalovirus (CMV) and herpes simplex virus (HSV) lytic phase, which are not as conserved, appear to regulate viral and host transcripts during lytic infection (Buck *et al.*, 2007;

Dolken *et al.*, 2007; Dunn *et al.*, 2005; Grey *et al.*, 2005; Pfeffer *et al.*, 2005). Nonconserved viral miRNA/mRNA interactions also exist that significantly affect the viral cycle. For example, EBV miR-BART-16, miR-BART-17-5p, and miR-BART-1-5p modulate the expression of EBV latent membrane protein 1 (LMP1), preventing LMP1-induced apoptosis in infected cells (Lo *et al.*, 2007). Therefore, viral miRNAs might mediate both conserved and newly acquired biological functions to which viruses have recently adapted.

Some viral miRNAs regulate the expression of host genes influencing host cell behavior. KSHV miRNAs suppress thrombospondin expression to enhance angiogenesis and induce resistance to TGFβ signaling (Samols *et al.*, 2007). hCMV miR-UL-112 silences MHC1-related chain B (MICB) and A (MICA) to evade NK cell recognition (Stern-Ginossar *et al.*, 2007).

9. SOME VIRAL miRNAs MIMIC HOST miRNAs

Other viral miRNAs mimic host miRNAs. KSHV miR-K12-11 closely resembles cellular miR-155, sharing the first nine 5 nucleotides including the seed region. This viral miRNA functionally mimics miR-155, as determined by microarray and functional studies (Gottwein *et al.*, 2007; Skalsky *et al.*, 2007). Since miR-155 is oncogenic and its overexpression leads to B cell lymphoma (Costinean *et al.*, 2006) and KSHV infection also causes B cell malignancies, it is likely that KSHV tumor induction may be linked, at least in part, to miR-K12-11 expression.

Other viral miRNAs with homology to known cellular miRNAs include EBV miR-BART5, rhesus lymphocryptovirus (rLCV) miR-rL1-8, and murine gammaherpesvirus 68 (MHV68) miR-M1-7-5p, all of which share perfect seed homology with cellular miR-18a, encoded by the miR-17–92 cluster, which is overexpressed in lymphomas and other cancers (Volinia *et al.*, 2006). Overexpression of this cluster in mice leads to lymphoma and autoimmunity (He *et al.*, 2005; Xiao *et al.*, 2008). Since miR-18a appears to silence CDKN1A (p21) (F. Petrocca, unpublished results), a key regulator of cell cycle progression and a central hub for a variety of tumor suppressor pathways, miR-BART5 may disrupt cell cycle control in EBV-infected cells and contribute to EBV-driven lymphomas and nasopharyngeal carcinomas.

10. HOST miRNAs ALSO REGULATE VIRAL REPLICATION

In primitive organisms that lack adaptive immunity, including plants, flies, and worms, RNAi is an important antiviral host defense mechanism (Li and Ding, 2006). Whether RNAi is important for mammalian antiviral

defense is still unclear. Several cellular miRNAs have been shown to target viral sequences, inhibiting viral replication. For example, impairment of the RNAi pathway by Drosha/Dicer knockdown enhances VSV replication, an effect that has been attributed to miR-24 and miR-93 downregulation (Otsuka *et al.*, 2007). Some host miRNAs may be essential for viral replication. For example, hepatocyte expression of miR-122, which binds to a seed-matching region in the hepatitis C virus (HCV) 5′-NCR, is needed for HCV replication by an unclear mechanism (Jopling *et al.*, 2005). Viruses can also reshape host miRNA expression to downregulate miRNAs that inhibit their own replication. For example, latent EBV infection of B cells leads to upregulation of both miR-155 and miR-146, the latter via NF-κB activation by LMP-1 (Lu *et al.*, 2008). HIV-1 infection downregulates expression of miR-17-5p and miR-20a in $CD4^+$ T cells. These miRNAs may inhibit HIV-1 replication by silencing PCAF, a transcriptional cofactor of Tat that is essential for viral replication (Triboulet *et al.*, 2007).

11. PROSPECTS FOR MANIPULATING miRNA FUNCTION FOR IMMUNOMODULATORY THERAPY

Since miRNAs have such a profound effect on immune function and response to pathogens, manipulating miRNA function in immune cells could potentially have therapeutic benefit. This approach might be able to alter expression of multiple genes at once to regulate key functional networks. miRNA function can in principle be augmented by transducing cells with double-stranded miRNA mimics that resemble the Dicer-processed endogenous miRNA or antagonized by ASO. The main obstacle to miRNA-based immune therapeutics is the challenge of transducing immune cells with nucleic acids *in vivo* (de Fougerolles *et al.*, 2007). Even *in vitro* transduction of lymphocytes is challenging. Manipulating miRNA function will likely be easier to accomplish for dendritic cells and macrophages to modulate the early steps of antigen recognition, innate immunity, and inflammation. Situations where local, as opposed to systemic, intervention could prove beneficial would also pose less of a delivery hurdle. The decade of drug development for antisense therapeutics has identified ways for *in vivo* transduction of some cells, including possibly myeloid antigen-presenting cells, with single-stranded oligonucleotides (as could be used to antagonize miRNAs).

Some progress has been made in developing methods for *in vivo* transduction of primary lymphocytes with siRNAs, which have the same chemical properties and pose the same delivery challenge as miRNAs. These involve targeted delivery of antibody fusion protein–RNA complexes (Song *et al.*, 2005) or antibody-coated siRNA-encapsulating

liposomes (Peer *et al.*, 2008). Fusion proteins have even been developed that induce silencing specifically only in activated lymphocytes by taking advantage of activation-associated changes in cell surface integrins (Peer *et al.*, 2007). Targeted intravenous delivery of antiviral siRNAs to primary lymphocytes was able to control HIV infection in humanized mice (Kumar *et al.*, 2008). Targeted delivery of cyclin D1 siRNAs encapsulated into β7-integrin-targeted liposomes administered intravenously was able to suppress experimental colitis by inhibiting T cell proliferation and cytokine production (Peer *et al.*, 2008). These encouraging examples suggest that the obstacles for therapeutic manipulation of miRNA expression in immune cells, although considerable, may be tractable.

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