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MicroRNA in the immune system, microRNA as an immune system

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Summary

The advent of microRNA has potentially uncovered a new level of complexity to be considered for every biological process. Through the modulation of transcription and translation, microRNA alter the basal state of cells and the outcome of stimulatory events. The exact effect of the microRNA network and individual microRNA on cellular processes is only just starting to be dissected. In the immune system, microRNA appear to have a key role in the early differentiation and effector differentiation of B cells. In T cells, microRNA have been shown to be key regulators of the lineage induction pathways, and to have a strong role in the induction, function and maintenance of the regulatory T-cell lineage. MicroRNA are also important for regulating the differentiation of dendritic cells and macrophages via toll-like receptors, with responsibilities in suppressing effector function before activation and enhancing function after stimulation. In addition to regulating key processes in the immune system, microRNA may also represent an archaic immune system themselves. Small interfering RNA of viral origin has been shown to function as an intracellular mediator in the suppression of viral infection in eukaryotes as diverse as plants, insects, nematodes and fungi, and there is growing evidence that endogenous mammalian microRNA can have similar impacts. In this article we speculate that the anti-viral function of microRNA drove the expression of different subsets of microRNA in different cellular lineages, which may have, in turn, led to the myriad of roles microRNA play in lineage differentiation and stability.

Keywords: B cells; dendritic cells; innate immunity; microRNA; regulatory T cells; T cells

Introduction

MicroRNA (miRNA) are small untranslated RNA species, highly conserved between different eukaryotic species.[1,2] MicroRNA are encoded in genomic clusters and produced by an elaborate expression and processing mechanism.[3] Following expression of the primary transcript by RNA polymerase II and III, nuclear processing by the enzyme Drosha produces a pre-miRNA transcript which can be shuttled into the cytoplasm.[4–9] Final production of the mature miRNA species requires further cytoplasmic processing by an RNase III enzyme, Dicer, producing a 19- to 24-base-pair product, capable of being incorporated into the RNA-induced silencing complex (RISC) which contains another core component, Argonaute (Ago) protein.[8,10–15] The RISC, in turn, is able to use the 'seed sequence' of the miRNA to recognize complementary messenger RNA (mRNA) transcripts for degradation or translational silencing.[11,16,17] The complexity of the miRNA network, with diverse effects on multiple mRNA species, has slowed down the dissection of their function. It is, however, apparent that miRNA have a fascinating role both within the immune system and as an immune system.

MicroRNA in the immune system

MicroRNA have long been known for their role in organ development, cellular differentiation, homeostasis and functioning.[18] More recently, studies conducted by many

Table 1. Studies of microRNA in adaptive and innate immunity

	Adaptive			
	B cells	Conventional T cells	Regulatory T cells	Innate
Whole network	Dicer[20]	Dicer[33,34,36]	Dicer[36,39,40]	
	Argonaute 2[19]	Drosha[38]	Drosha[38]	
Single miRNA	miR-17∼92[21,22]	miR-17∼92[20]	miR-146[36]	miR-125b[47]
	miR-150[24,25]	miR-101[37]	miR-150[36]	miR-146[49]
	miR-155[29–32]	miR-150[25]	miR-155[36,41–43]	miR-155[30,46,47]
	miR-181[23]	miR-155[29,30]		miR-223[44,45]
		miR-181[23,35]		

groups have demonstrated that miRNA are pivotal in both adaptive and innate immunity, including controlling the differentiation of various immune cell subsets as well as their immunological functions (Table 1).

MicroRNA in B cells

The essential role of miRNA in B-cell differentiation was first revealed in mice with a haematopoietic defect in *Ago2*, encoding an Ago protein indispensable for miRNA biogenesis and function.[19] Deficiency of Ago2 did not affect the generation of early pro-B cells, but significantly impaired further pre-B-cell differentiation and the succeeding peripheral B-cell generation. In agreement with this, a subsequent study where the whole miRNA network was ablated by employing the B-cell-specific deletion of a conditional allele of *Dicer* has demonstrated that B-cell differentiation is almost completely blocked at the pro- to pre-B-cell transition, at least partially as a result of the deregulation of a pro-apoptotic molecule, Bim.[20] Moreover, Dicer deficiency in B cells also resulted in sustained terminal deoxynucleotidyl transferase expression throughout B-cell maturation, altering the generation of the antibody repertoire.[20] Whereas these findings provided important insights as to how the miRNA network could impact B-cell differentiation and function, recent studies have begun to explore the role for individual miRNA in controlling different aspects of B-cell biology. For example, the aberrant Bim expression observed in Dicer-deficient B-cells has been attributed to the loss of miR-17∼92.[20] While the absence of miR-17∼92 leads to increased Bim expression and a developmental block at the pro-B to pre-B transition similar to that observed in Dicer-deficient B cells,[21] ectopic over-expression of miR-17∼92 results in enhanced B-cell proliferation and survival.[22] Similarly, miR-181 has also been shown to be a positive regulator for B-cell differentiation. Ectopic miR-181 expression results in a substantial increase in $CD19^+$ B cells accompanied with a reduction in T-cell numbers.[23] Moreover, miR-150 has also been shown to profoundly affect early B-cell differentiation and mature B-cell responses. The miR-150 is generally expressed at low levels in early B-cell progenitors, and ectopic miR-150 expression creates a developmental block at the pro-B to pre-B transition by targeting the transcription factor c-Myb. Importantly, in addition to miR-150 and c-Myb displaying opposing expression patterns, c-Myb-deficient mice and miR-150 over-expressing mice exhibit comparable phenotypes in B-cell differentiation.[24,25]

Unlike the miRNA discussed above, miR-155, which was frequently found highly expressed in B-cell malignancies in humans,[26,27] seems to control important aspects of B-cell biology without overly disturbing their early differentiation. While B-cell-restricted expression of a miR155 transgene leads to pre-leukaemic proliferation of B lineage cells, progressing to a severe B-cell malignancy,[28] no abnormal B-cell differentiation was reported on miR-155-deficient mice.[29–31] However, these cells did show an impaired capability to differentiate into germinal centre cells and undergo immunoglobulin class switching.[29] Although the precise mechanism as to how miR-155 impacts on germinal centre responses remains uncertain, impaired immunoglobulin class switching is probably the result of deregulated activation-induced cytidine deaminase (AID) expression. To this end, it was shown that mice with a disruption of the miR-155 binding site in the 3′ untranslated region of AID had a quantitative deregulation of its expression, along with functional consequences for class switching and affinity maturation similar to those observed in miR-155-deficient mice.[32]

MicroRNA in conventional T cells

Unlike B-cell differentiation, where there is a block in a specific developmental stage, the effect of T-cell-specific miRNA ablation is muted. In mice with a deficiency in Dicer in early T-cell progenitors (under the lck-driven cre transgene) the percentages of different double-negative, double-positive and the CD4–CD8 lineage (i.e. whether the transitioning cells became CD4 or CD8 single-positives) decisions also appears to be intact, albeit with a 10-fold reduction in total thymocyte numbers past the double-negative stage.[33] Alternatively, later deletion of *Dicer* with a CD4-drive cre transgene results in smaller

reductions in the number of total thymocytes, at the single-positive stage.[34] Although the precise molecular mechanism for the discrepancy between these studies is still not well characterized, the differential response in the numerical impact on T-cell differentiation depending on the timing of *Dicer* excision suggests that miRNA do not have a non-redundant role in any specific developmental event, but rather create a delayed numerical reduction as the result of diminished proliferation and increased susceptibility to cell death.[33,34] This numerical loss of thymic and peripheral T cells may be mediated in part by loss of miR-17∼92, as ectopic expression of this miRNA cluster results in an expansion of both CD4 and CD8 T cells.[22] The best evidence for miRNA playing a role in specific developmental stages of T-cell differentiation is from miR-181, which, as well as reducing the number of T cells in haematopoietic over-expression systems,[23] increases the sensitivity of T-cell receptor signalling.[35] It does this through the down-regulation of multiple phosphatases involved in the attenuation of signal transduction events downstream of the T-cell receptor, and in doing so increases the efficiency of both positive and negative selection.[35]

In the periphery, the specificity of the role of miRNA is more obvious, with an important task in the generation of different T helper (Th) lineages and T-cell function. T cells lacking Dicer exhibit preferential Th1 induction under non-polarizing conditions,[34] and have significant impairments in Th17 induction and transforming growth factor-β-mediated regulatory T-cell (Treg) induction.[36] By contrast with the whole network deletion, deficiency in miR-155 specifically promoted Th2 induction under non-polarizing conditions.[29,30] In terms of T-cell function, miR-101 is highly important in the post-transcriptional modulation of Icos. In the absence of miR-101-mediated regulation, the expression of Icos on naïve T cells increases, causing an effector T-cell-like phenotype and resulting in autoimmunity.[37] Further roles for specific miRNA in T-cell differentiation and function are likely to be discovered upon further investigation.

MicroRNA in regulatory T cells

One subset of T cells that heavily rely on miRNA for generation and function are the Forkhead box p3 (Foxp3)-dependent Treg cells, including both thymic-derived and peripherally induced Treg cells. Both the thymic and peripheral induction of Treg cells is enhanced by miRNA. In the thymus, mice with either Dicer or Drosha ablated using the CD4-driven cre transgene have a reduction in thymic Foxp3$^+$ Treg disproportionate to that of other T-cell subsets.[36,38] Likewise, in the periphery miRNA also play an important role for the generation of adaptive Treg, as a lack of Dicer results in a dramatic reduction of Foxp3 induction in naïve T cells upon transforming growth factor-β stimulation, as discussed above.[36] This numerical paucity leads to spontaneous inflammatory disease later in life.[36]

In addition, miRNA play a pivotal role in controlling Treg function. Depletion of miRNA within the Treg lineage results in fatal autoimmunity indistinguishable from that in Treg-deficient mice.[38–40] Furthermore, while both the homeostasis and suppressor capacity of Dicer-deficient Treg cells were markedly reduced under non-inflammatory conditions, under inflammatory conditions Treg cells entirely lost their suppressive capacity and anergic profile.[40] These data implicate miRNA as key guardians of a stable Treg functional programme under conditions of lineage challenge. Although specific miRNA responsible for controlling Treg function remain to be identified, the reduced homeostasis of Dicer-deficient Treg cells appears to be caused by loss of miR-155. The miR-155 is directly regulated by Foxp3[41,42] and is critical for maintaining the heightened responsiveness of Treg cells to the key survival and growth factor, interleukin-2, through targeting suppressor of cytokine signalling 1 (SOCS1), ensuring their fitness in a competitive environment.[43] The miRNA that enhance suppressive function and lineage stability have not yet been identified.

MicroRNA in the innate immune system

Recently, several efforts have also been made to demonstrate the role of miRNA in innate immune cells. For example, miR-223 has been shown to promote granulopoiesis *in vitro*.[44] Moreover, mice lacking miR-223 develop more inflammatory lung lesions and tissue destruction upon endotoxin challenge as a result of hyperfunctional neutrophils.[45]

In macrophages and dendritic cells (DCs), miRNA have an important role in the maturation of cells into the active lineage through toll-like receptors (TLR). Stimulation with interferon-β and TLR ligands causes miR-155 induction, via both the nuclear factor-κB pathway and Jun N-terminal kinase pathway.[46,47] The implication of miR-155 being involved in the TLR-induced antigen presentation pathway was confirmed by a study showing that miR-155-deficient DCs are unable to induce efficient T-cell activation in response to antigens because of impaired antigen presentation capacity and costimulation activity.[30] SOCS1 is a bona fide miR-155 target,[43] so it is worth noting that SOCS1 negatively regulates the antigen-presenting capacity of DCs.[48] Deregulation of SOCS1 in the absence of miR-155 could therefore account for the impaired DC function. The miR-146 is also involved in the TLR signalling loop. Both miR-146a and miR-146b are transcriptionally up-regulated after lipopolysaccharide (LPS) stimulation, but only mature miR-146a is generated (providing another example of the complex nature of the regulation of miRNA expression).[49] The increase in

miR-146a expression reduces the expression of two key components (IRAK1 and TRAF6) of the TLR signalling cascade.[49] The miR-146a therefore appears to function as an effector molecule in driving a negative feedback mechanism to attenuate the TLR response, preventing excess inflammation. In contrast to miR-155 and miR-146, in macrophages miR-125b is down-regulated upon LPS stimulation.[47] Tumour necrosis factor-α (TNF-α) is a target gene of miR-125b, this suggests that down-regulation of miR-125b is required to ensure that a proper inflammatory response is generated by macrophages in response to microbial stimuli, or conversely miR-125b could be considered to be a safety mechanism to ensure negligible TNF-α expression by inadequately stimulated macrophages.

MicroRNA as an immune system

In addition to the vital role of miRNA in co-ordinating the appropriate behaviour of the immune system, miRNA is also capable of acting as an intracellular immune mediator. With short seed sequences and tolerance for mismatches, the wide array of miRNA will include those capable of recognizing and down-modulating viral RNA stability and translation. This property grants miRNA the capacity to shut down transcription of viral mRNA required for successful proliferation and, in the case of cytoplasmic viruses, directly attack viral genomes.

Anti-viral microRNA

The RISC complex, by using small interfering RNA (siRNA) produced by the cleavage of double-stranded viral RNA, has a capacity to suppress viruses that is conserved across most eukaryotic lineages, including plants,[50] insects,[51] nematodes[52,53] and fungi.[54] More controversially, evidence has recently grown for the capacity of endogenous small RNA species, miRNA, to also suppress viral proliferation via incorporation into the RISC complex. In mammals, the repression of Dicer, and hence miRNA production, creates hypersensitivity to human immunodeficiency virus type 1 (HIV-1), vesicular stomatitis virus (VSV) and influenza A virus.[55–57] For miRNA to act directly upon viral targets requires the fortuitous complementarity of cellular miRNA seed sequences with viral sequences. The number of established cases of miRNA-mediated direct virus suppression in mammals is still relatively low (Table 2); however miRNA have been shown to impact on the proliferation of hepatitis C virus (HCV), HIV-1, human cytomegalovirus, primate foamy virus type 1 (PFV-1) and VSV.[56,58–61]

One explanation for the inhibition of viral proliferation by miRNA is simply the coincidental recognition of a few viruses by endogenous miRNA seed sequences. An alternative explanation is that miRNA-RISC is the anticipatory version of the siRNA-RISC mechanism, and thus constitutes a legitimate arm of the immune response. While insufficient data-points are available to discriminate between these alternative explanations, an analysis of 228 human viruses predicts that 62 viruses from six different viral families include target sites for human miRNA.[62] Perhaps the most suggestive evidence for the latter explanation is the extent to which viruses have evolved mechanisms to evade miRNA. PFV-1, which is sensitive to miR-32, includes a protein, Tas, that suppresses miRNA function[61] (although this has been disputed).[63] Likewise, HIV-1, which includes recognition sites for multiple human miRNA, has several mechanisms to evade this host defence, including RNA sheathing and quenching of the miRNA machinery.[55,64–66] Viruses have even co-opted miRNA genes to use against the host, creating an environment more conducive to survival.[67]

Immunity and identity

If miRNA do indeed represent a legitimate innate immune system mechanism, there is one significant drawback in miRNA as an immune mediator – the inability for rapid evolution. The anticipatory antigen receptors, T-cell receptor and antibody, include the random generation of recognition to counter the rapid evolution of viruses. The innate antigen receptors recognize conserved structural features of microbes that do not exist in the host, such as the binding of dsRNA and LPS via the TLR. MicroRNA, however, recognize a common component to both microbe and host, relying instead on

Table 2. Direct suppression of mammalian viruses by endogenous microRNA

Host	Virus	MicroRNA	References
Human	HCV	miR-1, miR-30, miR-128, miR-196, miR-296, miR-351, miR-431 and miR-448	58
Human	HIV-1	miR-28, miR-125b, miR-150, miR-223 and miR-382	59
Human	HMCV	miR-100 and miR-101	60
Human	PFV-1	miR-32	61
Mouse	VSV	miR-24 and miR-93	56

HCMV, human cytomegalovirus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; PFV-1, primate foamy virus type 1; VSV, vesicular stomatitis virus.

sequence specificity. The potential problem created by this is that modification of the miRNA seed sequences to counter change in the virus could have catastrophic effects on the transcriptional profile of the host cell. This problem does not occur because miRNA have a high conservation rate.[1] Hence, to be effective as an immune mediator, cells need to carry miRNA that cover a broad set of seed sequences, such that viral evolution to escape recognition by miRNA becomes infeasible. In this regard, miRNA would act in an anticipatory manner, containing pre-existing miRNA that are likely to include recognition sequences against viruses with which the host has not had previous experience. The potential for broad viral recognition by miRNA is supported by the prediction of human miRNA with seed sequences capable of recognizing invertebrate-specific viral genomes, against which there is no evolutionary pressure to create specific responses.[62] As with other arms of the innate immune response, viruses specialized to a host will have evolved mechanisms to mitigate the immunological effects of miRNA in at least one cell type, but non-specialized viruses, or specialized viruses infecting tissue outside their evolved tropism, may be restricted because of recognition by miRNA.

In this regard, miRNA as an immune mediator in eukaryotic cells represents convergent evolution to the restriction-modification system in prokaryotic cells. Although generally only considered with regard to the type II restriction enzymes used as a tool in molecular biology, restriction-modification systems comprise a broad immune mediator, with over 3500 restriction-modification systems of four main classes being found across bacteria and archaea. Restriction-modification systems generally consist of a sequence-specific restriction component (endodeoxyribonuclease), which cleaves unmodified DNA, and a sequence-specific modification component (typically DNA methyl-transferase), which protects the host DNA.[68] Like miRNA, the restriction-modification system recognizes short nucleotide sequences and neutralizes the nucleotide which contains it. A bacteriophage (bacterial virus) containing the unmodified recognized sequence has a 10^3–10^4-fold decrease in survival rate.[68] As with the virus–miRNA relationship, numerous bacteriophages have evolved means of reducing susceptibility, such as selection against target sequences or by carrying enzymes that inactivate restriction (e.g. RaI from Phage l inactivates EcoK12) or mimic modification (e.g. *Bacillus* phage p11 broadly methylates its genome).[68,69] Also analogous to the virus–miRNA relationship, certain bacteriophages have co-opted restriction-modification systems into their own genomes, for use against the host.[68] In what may yet prove to be a lesson to those studying virus–miRNA relationships, counter-subversion by bacteriophage escape also occurs, with enzymes such as *Diplococcus pneumoniae* Dpn1 and *Escherichia coli* Mcr, which specifically cut the modified sequences of bacteriophages endeavouring immune evasion.[68]

Of relevance in the current context, a key requirement for the efficacy of the restriction-modification system as an immune mediator is cell-to-cell diversity. In a homogeneous bacterial population a given restriction-modification system may reduce the survival rate 10^3–10^4-fold upon infection of the first cell; however, surviving bacteriophages have the correct genomic modification and reach normal survival upon subsequent infection of new hosts.[68,69] Likewise, in a eukaryotic system in which each cell in an individual contained the same miRNA profile, any virus with the correct genomic composition to survive in one cell would contain the potential to survive in all cells within the host. For this reason, the evolution of miRNA as an immune mediator in multicellular organisms would select for different cell types containing differential miRNA profiles, with the prediction that miRNA expression patterns would influence tropism.[70]

For several viruses, a miRNA-dependent tropism effect has been recorded. For example, the engineering of a neuronal miRNA recognition site into polio virus alters the ability of the virus to proliferate in neural tissue.[71] Likewise the miRNA profile of resting $CD4^+$ T cells (including miR-28, miR-125b, miR-150, miR-223 and miR-382) is effective in inhibiting HIV-1 proliferation, whereas that of the closely related cell type, activated $CD4^+$ T cells, is not.[59] An interesting example is HCV, where the virus has adapted to the miRNA profile of homeostatic hepatocytes, using miR-122a during replication.[72] Despite this specialization, the virus has profoundly reduced success when the miRNA profile of hepatocytes is modulated upon interferon-β exposure, with down-regulation of miR-122 and up-regulation of miR-1, miR-30, miR-128, miR-196, miR-296, miR-351, miR-431 and miR-448.[58] If, as we have speculated here, miRNA evolved as an immunological mechanism, the unfolding role of miRNA in developmental and cell differentiation decisions may have been a direct consequence of the immunological mechanism. By selecting for differential miRNA profiles in each cell lineage, an immunological mechanism could create a lineage buffer between different cells types, as fluctuation in basal transcription become smoothened by the actions of miRNA.[73] Hence, in the case of regulatory T cells, the miRNA signature buffers the cell type from committing to alternative lineages when exposed to environmental pressures.[40] In turn this necessitates changes in the miRNA profile during lineage and developmental decisions, creating critical subsequent developmental functions for miRNA after the adoption of the original immunological function.

Concluding remarks

If the original evolutionary function of miRNA was to operate as a eukaryotic anti-viral defence network, the

analogy of restriction-modification systems in bacteria and archaea indicates that there would have been a strong selection for diversity of expression in different cell lineages. In this context, it is interesting that so many of the key functions of miRNA identified to date lie in development and differentiation. In B cells the strongest roles of miRNA appear to lie in the lineage-change decisions of the pre- to pro-B-cell transition and the naïve to germinal centre transition. Likewise in T cells, the strongest identified role appears to be in the lineage-change decision of which effector lineage to enter upon activation. In DCs miRNA again appear to play a strong role in the lineage-change decision of TLR-induced maturity. We would anticipate that further investigation of lineage-change decisions will illuminate further fascinating roles for miRNA.

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