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MicroRNA control of lymphocyte differentiation and function

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Summary

MicroRNAs (miRNAs) are a class of endogenous, non-coding regulatory RNAs that control gene regulation by guiding silencing protein complexes to mRNA in a sequence-dependent manner. In this way miRNAs are able to repress gene expression post-transcriptionally by affecting mRNA stability or translation. These ubiquitous molecules play central roles in a wide range of biological processes, including cell proliferation, differentiation and apoptosis. Within the context of the immune system, genetic studies have identified distinct roles for specific miRNAs in gene regulation during development, activation and maturation. Conversely, dysregulation of miRNA expression has been specifically correlated with cancer. This review outlines our current understanding of miRNA function in lymphocytes as it impacts expression of protein-coding genes in the context of proper development, as well as oncogenesis.

Introduction

Non-coding, regulatory RNAs are an emerging class of molecules that play important gene-regulatory roles in animals and plants. microRNAs (miRNAs) comprise the better understood subset of this diverse and nearly wholly uncharacterized set of regulators. miRNAs are ~22nt-long small, guide RNA that associate by imperfect sequence complementarity with their target mRNAs, usually (though not exclusively) within the 3'untranslated region (UTR) [1,2]. This association is thought to shape gene expression profiles both by mediating mRNA decay and by interfering with translation [1].

Unlike the case in lower organisms, where these small RNAs lead to almost complete transcriptional silencing, in vertebrates, miRNAs are better thought of as rheostats of gene expression, leading to the fine tuning (rather than complete shut-off) of protein levels in the cell [3]. miRNA targeting of a particular mRNA rarely yields more than a 3-fold decrease in transcript levels, and this rather minor differential appears to be mirrored at the translational level, as has been shown by global comparisons of transcript levels (using RNAseq) vs. ribosomal profiling [4]. However, most mammalian mRNAs contain miRNA targets that have been conserved through evolution (creating a useful tool for target identification by bioinformatics approaches [1]). This contrast between conservation of target sites yet modest reduction in protein output imparted by each individual miRNA:mRNA interaction is, at the moment, a mystery.

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To study global miRNA regulation in lymphocytes, immunologists have studied gene expression outcomes by monitoring protein changes in animals where miRNA biogenesis has been compromised (e.g. conditional Dicer mutants). Additionally, multiple studies of specific miRNA: function have been undertaken, either by ablating or by overexpressing the miRNA of interest in a controlled fashion (knockouts and knockins), although in such cases the possibility of indirect effects can be difficult to rule out. Finally, approaches that disrupt only specific miRNA: target interactions (either by mutating a target site within a specific 3'UTR or by using antisense reagents to hybridize to the target site and prevent miRNA pairing) have also been attempted. Below, we will summarize the contribution of these approaches to our understanding of how these small RNAs contribute to lymphocyte development and homeostasis.

Global deletion of microRNAs and microRNA profiling in lymphocytes

One of the approaches to understand the control of lymphocyte function by miRNAs has been the generation of animal models in which global miRNA maturation is blocked through the deletion of Dicer endonuclease. Dicer plays a crucial role in miRNA biogenesis by cleaving pre-miRNAs to generate a double strand RNA duplex that contains the mature miRNA. Dicer deletion in the mouse germline has a lethal phenotype, but conditional Dicer alleles have allowed addressing its role in specific cell lineages.

Early elimination of Dicer in the T cell lineage mediated by lck-Cre expression causes a dramatic reduction of thymocyte and peripheral T cell numbers, presumably due to increased apoptosis [5]. In addition, mature T cells generated in Dicer^{fl/fl} CD4-Cre animals, which promotes Dicer elimination in double positive thymocytes, show a preferential bias to Th1 differentiation [6] and a defective generation in regulatory T (Treg) cell development [7]. The role of Dicer in Treg development and function was further explored in three independent studies that made use of Foxp3-Cre BAC transgenic and Foxp3-Cre knock-in mouse strains [8–10]. Elimination of Dicer at this stage does not preclude Treg cell generation but interferes with their suppressor activity and promotes fatal inflammatory disease, very much resembling the phenotype observed in Foxp3 transcription factor-deficient animals. Notably, depletion of Dicer in Tregs gives rise to a much more dramatic phenotype than at early developmental stages, probably reflecting that the effect of Treg deficiency in the CD4-Cre Dicer model is somehow dampened by additional alterations in effector T cell subsets. More recently Dicer disruption by Tie2-Cre (expressed in hematopoietic and endothelial cells) has been shown to disrupt the development of natural Killer T (NKT) cells, another subset of regulatory cells in the immune system [11].

The role of Dicer in the B cell lineage has been explored in two independent analyses. In the first one Dicer was eliminated with the mb1-Cre strain [12] at the earliest stage of B cell differentiation, which promoted an almost complete block at the pro- to pre-B cell transition due to massive apoptosis of this latter subset. This phenotype is at least partially due to aberrant regulation of the pro-apoptotic Bim protein, a target of miR-17~92 (also known as oncomiR-1, see below) in Dicer deficient pro-B cells. Consistently, counteracting Bim activity by Bcl-2 overexpression partially rescues the pro-B cell block [12]. Of note, deficiency in miR-17~92 promotes a similar B cell block and Bim accumulation [13], supporting the view that this miRNA is one of the major contributors to the phenotype observed in this Dicer deficient model. In a second model, Dicer depletion at later B cell developmental stages achieved with the CD19-Cre mouse strain caused a biased generation of marginal zone (MZ) versus follicular (FO) B cell spleen subsets. This differentiation phenotype is accompanied by a skewed BCR repertoire, accumulation of self-reactive antibodies and autoimmunity. miRNA profiling of MZ vs FO cells and functional analysis

allowed to identify miR185 as a critical player in this phenotype through the regulation of BCR signaling by Btk [14].

Together these reports indicate that Dicer-dependent miRNA generation is essential at a number of developmental and functional stages in lymphocytes. Although efforts have been made to single out specific miRNAs contributing to the phenotypes observed in these models [12,14], these have been hindered by the difficulty to reconstitute them with candidate miRNA transgenes, as their expression would normally require Dicer-dependent processing as well. However, the recent finding that maturation of a particular miRNA, miR-451, is independent of Dicer [15,16], can help to bypass this issue. Indeed, miR-451's backbone has been shown to reprogram miRNA processing [17], thus providing a valuable tool for transgenic rescue of Dicer conditional models with candidate miRNAs.

In addition, defining miRNA expression profiles in specific cell lineages is a most useful aid to identify miRNAs potentially relevant to their development or function. A number of early analyses approached miRNA profiling in particular lineages of the immune system by microarray hybridization or sequencing of miRNA libraries (reviewed in [18]) and a major contribution was made by the sequencing of 98 small RNA libraries of hematopoietic origin [19]. Another miRNA library sequencing study was focused on identifying the human mature B cell miRNome, reporting the expression of at least 66 new miRNAs in this lineage [20]. Very recently, a combination of next generation sequencing approaches has shown that miRNA expression is tightly regulated by epigenetic, transcriptional and post-transcriptional mechanisms during mouse lymphopoiesis [21]. We are looking forward to additional high-throughput technologies that match these miRNAs to their cognate transcripts *in vivo* thus obviating the need to use bioinformatic algorithms. One such approach is the promising Ago-HTS-CLIP, which allowed the identification of functional protein-RNA interaction sites by high-throughput sequencing of RNAs isolated by crosslinking immunoprecipitation [2].

Individual miRNAs that control T cell differentiation and function

Different miRNAs have been identified that play a role in T cell differentiation and function (Table 1). miR181a expression was found to be tightly regulated during intrathymic T cell development and to modulate the T cell antigen receptor (TCR) response, most likely through the downregulation of different phosphatases that usually dampen TCR signaling pathways. Thus, miR181a acts as a posttranscriptional modulator of TCR sensitivity thresholds and contributes to clonal selection events during T cell development in the thymus [22,23].

miR-155 is widely expressed in immune cells [21] and is functionally involved in different aspects of the adaptive immune system. Regarding T cells, it was initially found that miR-155 deficient animals display a bias towards Th2 differentiation [24,25]. In line with this role in Th cell lineage decisions, miR-155^{-/-} mice are highly resistant to experimental autoimmune encephalomyelitis (EAE) due to a defective Th17 and Th1 development, which supports a role for miR-155 in the inflammatory response [26]. Interestingly, miR-155 is also functionally relevant to Treg function, where its expression is regulated by Foxp3 [27]. Indeed, miR-155 maintains Treg cell proliferation and homeostasis most likely by modulating the sensitivity to IL2 through the downregulation of suppressor of cytokine signaling 1 (SOCS1) expression [27]. Therefore, miR-155 seems to regulate T cell (and B cell, see below) differentiation and function through a complex network of pathways that have not been completely elucidated.

Additional regulation of Treg function is provided by miR-146. miR-146 deficiency in Tregs results in a defect in their suppressor function and dysregulated IFN γ responses, seemingly

through an increase in signal transducer and activator 1 (Stat1) expression and activation [28]. Thus, miR-146 and miR-155 seem two important players in the fatal inflammatory disease observed in mice where Dicer was eliminated in Tregs (see above, [8–10]). T helper responses are regulated by additional miRNAs. miR-182 expression is induced in T helper cells by IL2 and allows to maintain the late phase of expansion of these cells by post-transcriptional regulation of Foxo1 transcription factor [29]. Blockade of miR-126 with antagomirs - a class of chemically engineered oligonucleotides used to silence endogenous microRNAs- diminishes the Th2 response and lessens allergic airways inflammation in an in vivo model, although the contribution of the innate immune system to this effect has not been clarified [30]. Gain- and loss-of-function analysis showed that Th17 cells are positively regulated by miR-326, a miRNA whose expression is upregulated in patients with multiple sclerosis, and that this regulation is critical in the development of EAE [31].

Individual miRNAs that control B cell differentiation and function

A number of individual miRNAs expressed in the B cell lineage have been shown to control B cell differentiation in the bone marrow or the activation and function of mature B cells (Table 1). An early study showed that miR-181 overexpression in hematopoietic bone marrow progenitors leads to an increase in the fraction of B cell lineage cells [32]. In addition, ectopic expression of miR-150 causes severe defects in B cell development due to a block at the pro- to pre-B transition, at least partially due to an increase in cell death rate [33,34]. Conversely, miR-150 deficiency promoted B1 cell expansion and an enhanced humoral immune response [33]. Interestingly, miR-150 was shown to target c-Myb, an essential transcription factor for B cell development and mice heterozygous for a c-Myb mutation displayed a block in pro- to pre-B differentiation, indicating that c-Myb is a major contributor of the phenotype observed in miR-150 transgenic mice [33].

A similar regulation point in B cell development has recently been shown by the axis miR-34a-Foxp1 transcription factor [35]. miR-34a is expressed at highest levels in pro-B cells and constitutive expression of miR-34a in a bone marrow reconstitution model revealed a reduction in the number of mature B cells caused by a block in the generation of pre-B cells [35]. Foxp1, a transcription factor required for early B cell development [36] is a direct target of miR-34a and its repression recapitulated the effects observed by miR-34a overexpression [35]. In summary, these studies show that a number of miRNAs, including miR-181, miR-17~92 (see above), miR-150 and miR34a are critical to B cell development and that they may achieve their effect through the regulation of a relatively limited number of key targets mainly involved in transcriptional regulation and cell death.

Regarding B cell activation and function, one of the key miRNA regulators seems to be miR-155. miR-155 is induced upon B cell activation in germinal centers although its expression is not restricted to this lineage ([21,25] and see above). Germline deletion of miR-155 promotes a reduction in germinal center B cell numbers [24,25] which was shown to be B cell autonomous and to stem at least partially from a deficient generation of plasma cells and affinity maturation [37]. miR-155 was initially shown to target PU.1 transcription factor, whose overexpression led to a similar defect in the generation of IgG1 switched cells [37]. miR-155 has also been shown to target AID, a master regulator of Ig diversification, in approaches that specifically modified its target site in the 3'UTR of AID [38,39]. In addition, miR-181b has also been demonstrated to target AID [40], therefore, the levels of AID appear to be exquisitely controlled by two miRNAs that possibly act at different stages of B cell activation ([38–41] and C-A Reynaud et al., this issue).

miRNAs in lymphoid malignancies

The first hint on the involvement of miRNAs in cancer came from numerous profiling studies that revealed specific miRNA expression signatures associated with particular human malignancies (for recent reviews see [42,43]). One of the first miRNA patterns detected in cancer was the overexpression of 6 miRNAs in lymphoma and several solid tumors, all belonging to the same transcriptional unit, which is known as oncomiR-1 or miR-17~92. An early report showed that enforced expression of miR-17~92 in hematopoietic stem cells from E μ -myc transgenic mice accelerated the generation of B cell lymphomas when transplanted into lethally irradiated recipient animals, presumably due to a decrease in apoptosis induced by c-myc in this model [44]. More recently, a functional dissection of this cluster revealed that one of its individual miRNA components, miR-19, is the main player of the accelerated lymphomagenesis observed in the E μ -myc model and that it mediates direct repression of Pten and activation of the Akt mTOR pathway [45]. Likewise, miR19 overexpression cooperates with an activated form of Notch in the induction of T-ALL in a precursor transplantation model, further supporting the role of the miR-17~92 cluster in promoting lymphomagenesis [46]. In contrast, lymphoid-specific miR-17~92 transgenic animals die prematurely with lymphoproliferative disease and autoimmunity but do not develop frank lymphomas [47]. This phenotype is due, at least in part, to repression of Pten and Bim exerted directly by miR-17~92 [47]. These slightly contradictory results probably reflect that miR-17~92 overexpression contributes to but is not sufficient to promote lymphomagenesis and that the short lifespan of miR-17~92 transgenic animals prevents the accumulation of additional mutations that would be required for lymphoma development [41,47]. miR-155 expression has been also detected in various lymphoid malignancies, including CLL and DLBCL [43]. Importantly, miR-155 overexpression in vivo through an E μ /V_H miR-155 transgenic model promotes the generation of transplantable ALL/high grade lymphoma in which Ship and C/EBP β downregulation by miR-155 could be functionally relevant [48,49]. In addition, miR-29, a miRNA whose expression has been linked to B-CLL, induces the generation of a CD5⁺, B-CLL-like disease when overexpressed in a transgenic mouse model [50]. Finally, miR-21 has been shown to promote pre-B-cell lymphoma in an inducible transgenic model. Interestingly, subsequent miR-21 inactivation resulted in the regression of the tumors, thus presenting the first case of oncogene addiction to a miRNA, or oncomiR addiction [51]. Regarding miRNAs with a potential tumor suppressor activity, the best characterized example is provided by the miR-15a/16-1 cluster, which is located in a region deleted in 68% of B-CLLs ([52], reviewed in [43]). Targeted deletion of this cluster in the mouse resulted in lymphoproliferation with an indolent disease course that recapitulates many of the phenotypes associated with B-CLL in humans [53].

Concluding remarks

The uncomfortable notion that minor differences in protein expression can lead to significant consequences at the cellular level has led a number of investigators to propose that miRNAs can target multiple transcripts at once, thereby modulating the activities of entire pathways and amplifying individual effects many-fold. This notion can be illustrated by miR-155, which has been shown to have pleiotropic effects in different cell lineages and through different mRNA targets. However, most experiments performed thus far have reported specific targeting of a very small number of mRNAs (often one), thus validating the gene-ablation approach. Conversely, it is possible that only a handful of miRNAs are “master” regulators of gene expression in certain tissues. Undoubtedly, teasing apart miRNAs that are key regulators of protein expression from those who perform the grunt-work of keeping protein levels steady, will be the focus of future work.

Finally, additional modes of regulation that involve these small RNAs may be possible: through their targeting of 3'UTRs they could also regulate aspects of mRNA metabolism, including nuclear export, cytoplasmic localization, translational efficiency and mRNA stability specific to each transcript. This notion of alternative outcomes of miRNA regulation is an outstanding question that immunologists are well positioned to tackle.

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Table 1

Summary of the microRNAs relevant to lymphocyte differentiation and function discussed in the text.

	miRNA	Function	Targets	References
T cells	Differentiation	miR-181a	Modulates TCR signaling regulating clonal selection during T cell development in the thymus	DUSP5 DSP6 SHP2 PTPN22 [22,23]
		miR-155	Involved in Th lineage decisions: knockout mice show increased Th2 cell generation and impaired Th17 and Th1 generation	[24-26]
	Function	miR-155	Maintains Treg proliferation and homeostasis	SOCS1 [27]
		miR-146	Knockout mice show defects in Treg suppressor function and IFN γ response	Stat1 [28]
		miR-182	Maintains the late phase of expansion of T helper cells	Foxo1 [29]
		miR-126	Modulates Th2 response and inflammation mediated by allergic airways	[30]
		miR-326	Positively regulates Th17 cells	[31]
		miR-181	Favors B cell lineage differentiation	[32]
B cells	Differentiation	miR-150	Ectopic expression blocks B cell development at pro-B stage while knockout mice show B1 cell expansion and enhanced humoral immune response	c-Myb [33,34]
		miR-34a	Constitutive expression blocks B cell development at pre-B stage	Foxp1 [35]
	Function	miR-17-92	Knockout mice show a block in B cell generation at pro-B stage	Bim [13]
		miR-155	Regulates different aspects of B cell activation	PU.1 AID [24,25,37-39]
		miR-181b	Regulates germinal center reaction	AID [40]