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MicroRNA regulation of CD8+ T cell responses

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Abstract

MicroRNAs (miRNAs) are a class of short noncoding RNAs that play critical roles in the regulation of a broad range of biological processes. Like transcription factors, miRNAs exert their effects by modulating the expression of networks of genes that operate in common or convergent pathways. CD8+ T cells are critical agents of the adaptive immune system that provide protection from infection and cancer. Here, we review the important roles of miRNAs in the regulation of CD8+ T cell biology and provide perspectives on the broader emerging principles of miRNA function.

Keywords

MicroRNA (miRNA); cytotoxic T cell; T cell memory; T cell exhaustion

Introduction

The vertebrate immune system is a finely tuned and remarkably flexible instrument of host defense. A combination of innate pathogen associated molecular pattern recognition and the astonishing diversity and specificity of adaptive immunoreceptors provides protection from omnipresent and ever-changing pathological insults. From viruses to bacteria to eukaryotes to cancer, the diversity and abundance of potential threats that we are constantly encountering is a testament to our immune system's protective capacity.

CD8+ T cells are adaptive immune cells with an exceptional ability to specifically recognize and kill cells presenting foreign antigens (1). Each CD8+ T cell expresses just one of over a thousand trillion possible versions of the T cell receptor (TCR) with a unique specificity for an antigen that consists of a short peptide presented on class I MHC molecules by potential target cells (2). Upon encountering their cognate antigen, naïve CD8+ T cells become activated, undergo several rounds of cell division thereby generating clones of cells with the same receptor specificity, and differentiate to adopt a diverse multitude of specialized

behaviors depending on the context of their activation (1). The factors that govern these cell fates include the abundance of peptide-MHC antigen, TCR affinity for the antigen, the presence or absence of co-stimulatory signals, the local cytokine milieu, and cell-intrinsic factors such as transcription factors and epigenetic regulators of gene expression.

Among the cell-intrinsic factors that regulate T cell differentiation are microRNAs (miRNAs). miRNAs are short (21–24 nucleotide) noncoding RNAs that post-transcriptionally regulate target genes through interaction with their corresponding mRNAs (3). Functional expression of miRNAs is a complex process regulated by machinery that are regulated themselves, to an extent, by miRNAs (4). miRNAs are transcribed (mainly by RNA polymerase II) as long primary transcripts (pri-miRNAs) containing a hairpin structure that contains the mature miRNA sequence. These hairpins are cropped out of pri-miRNAs by the Microprocessor complex, consisting of the RNA binding protein DGCR8 and the RNase III family endoribonuclease Drosha, liberating precursor miRNAs (pre-miRNAs). Pre-miRNAs are exported from the nucleus via exportin 5 where they become accessible to a second RNase III, Dicer, which removes the pre-miRNAs hairpin loop, generating a short RNA duplex. One strand of this duplex becomes a mature miRNA upon its loading into an Argonaute (AGO) protein, forming a miRNA-induced silencing complex (miRISC) (3,4). The miRNA provides specificity to the miRISC, guiding AGO-mediated repression of target mRNAs via Watson-Crick base pairing between the miRNA "seed" sequence and "seed-match" sites found mainly within 3'UTRs. In this manner, miRNAs facilitate translational repression as well as deadenylation and degradation of their target mRNAs.

Adding to the complexity of miRNA regulation, the expression of miRNAs, their target genes, and the machinery required for miRNA function varies between cell types and differentiation states. Therefore, the role of a particular miRNA in one cell type can be dramatically different than its role in another. Depending on the expression level of a particular miRNA and a target RNA, the miRNA may exert little to no effect on protein abundance, tune protein abundance to appropriate levels, or even reduce protein abundance beyond a threshold necessary for effective function in the cell (5). These properties endow miRNAs with the ability to confer robustness to biological processes (6), and to buffer noisy and stochastic transcription that can be leaky and occur in bursts (7–9). Mathematical modeling supported by single-cell reporter assays confirmed a broad role for miRNAs in reducing noise in weakly expressed genes, while surprisingly increasing noise in highly expressed genes (10).

Like transcription factors, miRNAs mediate their biological functions through the regulation of networks of target genes. The magnitude of direct miRNA repression of an individual target mRNA and its corresponding protein is modest, almost never exceeding a two-fold effect. Nevertheless, individual miRNA:target interactions are biologically relevant, as suggested by the evolutionary conservation of many miRNA binding sequences, and supported by naturally occurring variants in miRNA binding sites that alter physiology or confer risk for pathology (11–13). In a few cases, the *in vivo* requirement for an individual miRNA:target interaction has been interrogated experimentally by mutating a binding sequence by gene targeting in mice (14–17). Each of these mutations was sufficient to produce a profound phenotype, yet none of them could account for the full effect of

removing the miRNA entirely. Indeed, all of them involve mutating a single binding site for the same miRNA (miR-155) in different target genes.

Exemplifying the broad nature of miRNA targeting, algorithms that take into account the conservation of miRNA seed-matches within 3'UTRs predict that over 60 percent of all RefSeq annotated genes can be regulated by one or more miRNAs (11). Biochemical methods that detect miRISC occupancy on mRNAs, such as AGO2 high-throughput sequencing of RNAs isolated by crosslinking immunoprecipitation (AGO2 HITS-CLIP or AHC) (18) and differential AHC, in which AHC data from cells sufficient or deficient for a particular miRNA are compared (19), generally support these predictions, but indicate that they likely underestimate the extent of miRNA:target interactions. Including all mRNAs with 3'UTR seed-matches, regardless of conservation, expands the list of predicted miRNA targets to include over 98 percent of all RefSeq annotated genes (Figure 1A). Prediction based on sequence alone is not sufficient evidence that targeting occurs. However, it can guide biochemical analyses of miRNA binding to transcripts, as well as expression analyses that measure the context-specific functional effects of miRNAs. Comparative measurement of nascent and mature mRNA levels and quantitative proteomics showed that only 99 targets were stabilized by miR-144/451 deficiency in erythroblasts (20). AHC supported these findings by demonstrating that for most transcripts with miR-144/451-dependent AGO2 binding, there was no appreciable stabilization in miR-144/451 deficient erythroblasts. Further work will be necessary to determine the generalizability of these findings among other miRNAs in different contexts. Importantly, a single RNA can be targeted by multiple miRNAs, and each miRNA influences the expression of tens to hundreds of direct target RNAs (Figure 1B). These targets often include sets of genes that function in common biological pathways, providing opportunities for miRNAs to produce additive phenotypic effects, and to control multiple potential limiting factors in noisy or dynamic gene expression programs.

miRNAs are required for the normal behavior of almost every vertebrate cell type in which their biogenesis or function has been experimentally disabled. In this review, we focus on the regulatory mechanisms that govern CD8+ T cell fate and function within immune responses, emphasizing the roles of miRNAs in controlling or modulating these mechanisms. Additionally, we provide commentary on emergent principles of miRNA regulation.

miRNA regulation of CD8+ T cell function

In response to activation by their cognate antigen, T cells adopt gene expression profiles conducive to rapid proliferation and the deployment of effector functions. While many of these changes occur at the transcriptional level, as much as 50 percent are mediated post-transcriptionally (21). miRNAs also exhibit profound changes in abundance in response to T cell activation owing at least in part to the rapid turnover of miRNA processing machinery and Argonaute proteins (22,23). miR-16, miR-142–3p, miR-150, miR-142–5p, miR-15b, and let-7f are the most abundantly expressed miRNAs in naïve CD8 T cells and all of these miRNAs are down-regulated with *in vitro* activation (24). Globally, the majority of miRNAs are immediately down-regulated in response to T cell activation (23). However, some, such

as miR-155, are transcriptionally upregulated and increase in abundance during T cell activation (25–27).

Essential to an effective cytotoxic T cell response is the proliferation and accumulation of sufficient quantities of antigen-specific cells capable of killing infected cells and cancer. CD8$^+$ T cells lacking miR-155 fail to appropriately expand in response to LCMV infection (28). In fact, in the absence of miR-155, there is a ten-fold reduction in antigen-specific CD8$^+$ T cell accumulation (29) and this appears to be driven by miR-155 effects on both proliferation and survival (30). Members of the miR-17~92 cluster of miRNAs are also up-regulated in response to CD8$^+$ T cell activation *in vivo* (31,32). Consistent with previous reports describing lymphoproliferative disease resulting from overexpression of the miR-17~92 cluster (33), the proliferative capacity of antigen-specific CD8$^+$ T cells is diminished in the absence of miR-17~92 (32). miR-17~92 has been demonstrated to directly target the tumor suppressor PTEN and the pro-apoptotic protein Bim, providing at least two targets with shared functionality by which miR-17~92 may act. miR-15/16 has been shown to directly target a network of cell cycle and survival associated genes including *Ccne1* and *Bcl2* (34–36). Consistent with these observations, deletion of miR-15/16 results in increased proliferative capacity and survival among antigen-specific CD8$^+$ T cells (36,37).

Highlighting the importance of miRNAs in restraining CD8$^+$ T cell effector function, Dicer-deficient CD8$^+$ T cells exhibit increased production of perforin, granzyme B, and interferon-gamma (IFN-γ) (38). T cell migration out of central lymphoid organs is a critical component to effector responses and is mediated by surface expression of S1P1 (39), which is inhibited by CD69 (40). Dicer-deficient CD8$^+$ T cells are defective in their ability to migrate out of central lymphoid organs and fail to accumulate in response to infection, likely due to retained surface CD69 expression (41). Several miRNAs that participate in this restraint are downregulated upon T cell activation, linking activating signals to diminishment of a miRNA barrier to T cell differentiation and acquisition of effector functions. Naïve T cells strongly express several members of the let-7 family of miRNAs. Inhibiting let-7 production by overexpression of the regulatory RNA binding protein LIN-28 leads to increased baseline CD8$^+$ T cell proliferation (42). Conversely, let-7 overexpression inhibited antigen-specific T cell clonal expansion and effector function. Retroviral over-expression of pri-miRNAs revealed that miR-139–3p can lead to downregulation of perforin and the transcription factor EOMES, while miR-150 indirectly regulated expression of the high affinity interleukin (IL)-2 receptor, CD25 (38). miR-29 is also down-regulated in response to T cell activation, and is capable of regulating the expression of IFN-γ both directly (43) and indirectly by targeting the transcription factors, T-BET and EOMES (9).

On the other hand, miR-155 deficiency in CD8$^+$ T cells results in reduced cytotoxicity (28) and effector cytokine production (29). In addition to its role in these effector functions, miR-155 enhances the responsiveness of CD8$^+$ T cells to the homeostatic cytokines, IL-7 and IL-15 (44) as well as IL-2 (30). *Socs1* knockdown rescues the decreased IL-2 responsiveness of miR-155 deficient T cells. Additionally, *Socs1* knockdown in CD8$^+$ T cells phenocopies the tumor-protective effect of miR-155 over-expression. However, while miR-155 plays a critical role in the responsiveness of CD8$^+$ T cells in both acute and chronic infection models, target site mutation indicated that *Socs1* repression by miR-155 is only

sufficient to mediate a measurable effect in chronic models (17). In addition to *Socs1*, miR-155 represses *Ship1* and *Ptpn2*, two other negative regulators of AKT and STAT5 signaling (44), among many other direct target mRNAs (19). Overexpression of miR-155 leads to a remarkably improved anti-tumor response by tumor-specific CD8^{+} T cells (30) suggesting that this approach may be beneficial in adoptive T cell therapies for cancer. The carefully dissected biology of miR-155 in T cell responses provides an edifying example of how miRNAs mediate pleiotropic effects through regulation of a network of target genes.

miRNA regulation of CD8^{+} T cell memory

In response to infection or cancer, antigen-specific CD8^{+} T cells may take on the properties of terminally differentiated effector (TE) cells armed with the ability to find and kill infected or abnormal cells, or those of memory precursor (MP) cells capable of persisting long after antigen clearance to provide protection from future encounters. In mice, TE and MP cells can be distinguished early in the immune response based on their surface expression of killer cell lectin like receptor G1 (KLRG1) (45) or the IL-7 receptor alpha chain, CD127 (46), respectively.

A single naïve CD8^{+} T cell has the potential to give rise to both TE and MP cells (47–49). Asymmetric cell division occurs during clonal selection of antigen-specific T cells, providing one plausible mechanism by which a single antigen-specific T cell could give rise to daughters with differing predetermined lineage fates (50). Noisy expression of lineage-determining factors may also promote stochasticity in these lineage decisions. Responding T cells exhibit a great deal of heterogeneity with respect to proliferative capacity, cytokine production, and the expression of KLRG1 and CD127, and these properties can be intrinsically biased by the TCR even as they are regulated by external cues (51).

No single master transcription factor regulates the differentiation of TE and MP cells, but many contributing factors have been identified. Positive regulators of MP cell differentiation include EOMES (52), TCF1 (53), ID3 (54,55), BCL-6 (56), STAT3 (56), FOXO1 (57,58), BACH2 (59), and MYB (60). Those found to bias cells towards TE fate include T-BET (45,61), BLIMP-1 (62,63), ID2 (50), STAT4 (64), and ZEB2 (65,66). Given that most of these lineage-biasing transcription factors only differ by approximately two-fold in their expression across TE and MP populations, it is likely that the fate of an activated CD8^{+} T cell is driven by the integration of their effects on downstream target gene networks (50).

Among memory CD8^{+} T cells, there is a great deal of further heterogeneity. T central memory (Tcm) cells, marked by high expression of CD127, CD62L, CD27, CXCR3, and CCR7, are more proliferative in response to antigen re-challenge. In addition, they tend to exhibit polyfunctionality with respect to effector cytokine secretion, producing IL-2, IFN-γ, and TNF. Conversely, they tend to express lower levels of the cytotoxin granzyme B. Due to their expression of the homing molecules CD62L, CXCR3, and CCR7, Tcm cells circulate through secondary lymphoid organs (SLOs), enhancing their probability of encountering antigen presenting cells (APCs) displaying their cognate antigen. CD27 is a member of the tumor necrosis factor receptor superfamily that acts as a co-stimulatory molecule on Tcm cells, but is absent from TE populations (67). Tcm cells provide superior anti-tumor

immunity (68,69) and protection against re-infection with virus (70,71). T effector memory (Tem) populations express high levels of CD127, but little or no CD62L, CXCR3, CD27, and CCR7. Instead, they often express chemotactic and adhesion molecules that allow entry into peripheral, non-lymphoid tissues while excluding them from SLOs. Functionally, Tem cells have enhanced killing capacity due to high expression of the inflammatory cytokines IFN-γ and TNF, as well as the cytotoxic molecules, perforin and granzyme B.

Defining the roles of miRNAs in TE and MP regulation is an active area of research. Several individual miRNAs, or families of miRNAs with identical seed sequences, are known to promote TE or MP differentiation. For example, we recently found that the abundant miR-15/16 family restricts the accumulation of MP cells (36). T cells lacking miR-15/16 exhibit early and sustained increases in MP cell production during the course of viral infection. In wildtype T cells, miR-15/16 bind and repress the expression of hundreds of target genes. Among these targets are a sizable network of memory cell associated genes, including *Il7r*, which encodes CD127. CD127 is required for the long-term survival of memory cells (72), and IL-7 availability can limit memory cell formation (73). Thus, miR-15/16 may restrict MP differentiation, proliferation and survival in part by tuning their expression of CD127. However, even transgenic over-expression of CD127 is insufficient to enforce memory cell differentiation (72), consistent with the expectation that others among the large number of potentially relevant miR-15/16 targets contribute to this phenotype (36). Of note, transfection of CD8$^+$ T cells with miR-15b mimics led to reduced apoptosis in response to stimulation with anti-CD3 *in vitro* and was attributed to the down-regulation of the programmed cell death-mediator, DEDD (74). These seemingly conflicting findings among *in vivo* and *in vitro* systems highlight the context-dependent nature of miRNA biology in T cells.

At the lower limits of gene expression, the fine tuning function of miRNAs can effectively enforce the silencing of genes with very low or leaky transcription (75). For example, miR-29 regulates IFN-γ production by Dicer-deficient CD4 T cells in part by silencing *Eomes*, a gene that can co-opt a transcriptional program usually enacted by another miR-29 target, T-BET (9). miR-29 overexpression in CD8 T cells reduces KLRG1$^+$ TE cell production while boosting the frequency of MP cells (9). miR-15/16 may help to enforce silencing of CD127 expression in TE cells, providing robustness to the restriction of IL-7 responsiveness to MP cells. CD127 is maintained at low levels in TE cells via GFI-1 mediated transcriptional silencing (76).

miR-155 increases CD8$^+$ T cell sensitivity to the common gamma chain receptor signaling cytokines, IL-7, IL-15, and IL-2 (30,44). As such, one might predict miR-155 to be a positive regulator of MP cell accumulation. Yet miR-155 is down-regulated in response to *in vitro* culture of CD8$^+$ T cells with IL-15, and miR-155 deficiency boosts the frequency of CD127$^+$ CD62L$^+$ IL-2 producing MP cells in response to infection with murid herpesvirus (77). In LCMV infection, miR-155 deficiency disrupts the formation of both MP and TE populations, while miR-155 over-expression enhances accumulation of Tem cells (78). Similar observations were reported by several groups, though their attribution of these phenotypes to direct miR-155 target genes varied. SHIP-1 was particularly implicated for its role in the negative regulation of AKT phosphorylation and T-BET expression (28).

However, once again it appears likely that miR-155 acts through a network of targets with additive or synergistic cell intrinsic effects on antiviral CD8$^+$ T cell responses (29).

The transcription factors ZEB1 and ZEB2 play reciprocal roles in the promotion of MP and TE cell differentiation respectively (79). ZEB2 is expressed among terminally differentiated cytotoxic T cells in a T-bet-dependent manner and plays a critical role in the suppression of MP associated genes while positively regulating TE-associated genes (65,66). Although the miR-200 family of miRNAs have been shown to negatively regulate both *Zeb1* and *Zeb2* in the context of epithelial differentiation (80), only *Zeb2 mRNA* appears to interact with, and be efficiently targeted by, miR-200 family miRNAs in T cells. Consistent with these findings, miR-200 was essential for normal memory CD8$^+$ T cell differentiation (79). This study documents the importance of cellular context to miRNA-target interactions, further highlighting the utility of empirical target identification by co-immunoprecipitation with AGO proteins.

Metabolism plays a critical role in the proliferation and differentiation of CD8$^+$ T cells. Inhibition of mTOR, for example, enhances memory cell persistence through the switch from glycolysis to fatty acid metabolism (81,82) and through the regulation of T-BET and EOMES (83). Over-expression of the miR-17~92 cluster of miRNAs enhances TE differentiation, while miR-17~92 deficient CD8$^+$ T cells are biased towards a MP phenotype (32). miR-17~92 regulates PI3K-AKT-mTOR signaling in T cells, and many other cell types, by targeting PTEN, a tightly tuned lipid and protein phosphatase that counteracts this signaling axis. These results do not rule out other targets as players. In fact, miR-17~92 also downregulates additional negative regulators of mTOR (e.g., *Pdcd1*, *Btla*, and *Fcgr2b*), though direct targeting and functional relevance of these putative targets has not been confirmed (31). The importance of functional testing of targets and consideration of the full suite of targets of a miRNA is highlighted by the case of mir-15/16, which regulates both mTOR and Rictor (37,84), but nevertheless restricts, rather than enhances, memory cell differentiation.

In human CD8$^+$ T cells, miR-143 overexpression enhanced the production of cells expressing the memory markers CD127, CD27 and CD28, whereas miR-143 inhibition reduced them (85). These effects were attributed to the miR-143 target *Glut1*, whose knockdown produced a phenotype consistent with that produced by miR-143 overexpression. The rate-limiting glycolysis enzyme HK2 was also down-regulated upon miR-143 overexpression (85), consistent with previous reports of direct targeting by miR-143 (86). Thus miR-143 coordinates the expression of at least two key targets in a pathway (glycolytic metabolism) critical to the regulation of CD8$^+$ T cell responses.

The roles of miRNAs in the transitions between memory cell states remains poorly understood. Transfer of CD62L^{hi} and CD62L^{lo} antigen-specific CD8$^+$ T cells supports a model of linear transition from naïve to Tem to Tcm cell identities (70). And KLRG1$^+$ CD127$^+$ Tem cells can lose KLRG1 expression and seed Tcm cell compartments in a *Bach2*-dependent manner (87). While over-expression of miR-150 decreases CD127$^+$ CXCR3$^+$ Tcm populations, subsequent reduced expression at later timepoints leads to an increase in Tcm cells (88). In some systems, miR-150 deficient memory T cells remained defective in

recall responses and cytolytic function (89). Overexpression of the miR-150 target MYB was sufficient to partially rescue some of the memory cell defects associated with miR-150 over-expression (88). This may be at least in part attributable to the indispensable role of MYB in regulating CD8$^+$ T cell stemness, memory, and polyfunctionality (60). Another study confirmed that miR-150 restrains memory CD8$^+$ T cell differentiation but attributed these effects to another direct target, *Foxo1*, a transcription factor that drives memory cell differentiation through induction of TCF1 (90). The established miR-150 target network contains several other functionally relevant target genes, including *Trp53* (91), *Slc2a1* (92), *Mtor* (93), *Ptrx7* (94), *Egr2* (95), in addition to indirect effects on CD25 expression (38).

miRNA regulation of CD8$^+$ T cell exhaustion

Effective memory generation requires the clearance of the pathogen or tumor. Persistent antigen exposure induces CD8$^+$ T cell "exhaustion", characterized by upregulation of inhibitory receptors including PD1, LAG3, and CTLA4, concomitant with reduced proliferation capacity, effector function and cell survival (96). Understanding the drivers and maintainers of T cell exhaustion is especially pressing in the context of tumor immunology. Over the two past decades, it has become evident that the reversal of T cell exhaustion can unleash existing tumor-specific cytotoxic T cells to attack and kill cancerous cells. Blocking inhibitory receptors can reverse exhaustion and induce productive antiviral and antitumor immunity (97–99). PD-1 blockade only temporarily reinvigorates exhausted CD8$^+$ T cells if the causative antigen is not cleared, indicating that targeting these surface receptors alone may be insufficient for many immunotherapies (100). Effective strategies for durably reprogramming exhausted T cells may improve existing and developing immunotherapies for cancer.

A growing number of transcription factors have been implicated in T cell exhaustion including T-BET, EOMES, Spry2, BLIMP-1, VHL, FOXO1, IRF, BATF, and NFATC1 (96,101) and more recently, the NR4A family members, NR4A1, NR4A2, and NR4A3 (102). Interestingly, many of these transcription factors are also critical to functional effector and memory CD8$^+$ T cells, suggesting a complexity to the drivers of exhaustion that remains to be fully understood. For example, IRF4 and BATF are essential for CD8$^+$ T cell effector function during infection with LCMV (103), but they can also elevate PD-1 expression and repress TCF1, thereby promoting exhaustion while inhibiting memory formation (101).

miRNAs also play a role in CD8$^+$ T cell exhaustion. In the context of cancer, tumor-derived TGF-β can lead to elevation of miR-23a expression, thereby downregulating BLIMP-1 and a loss of effector function (104). Functional inhibition of miR-23a led to improved effector function and a more durable response to established tumors. In response to chronic infection with LCMV clone 13, miR-31 deficient CD8$^+$ T cells express reduced levels of exhaustion markers and retain characteristics of effector cells, including production of cytotoxins and cytokines (105). Mice lacking miR-31 expression only in T cells were protected from the wasting associated with chronic infection and harbored lower viral titers. miR-155 overexpression enhances the persistence of exhausted CD8$^+$ T cells during chronic infection (106). Uncoupling persistence from effector function, miR-155 overexpression fails to restore cytokine production and cytotoxic potential, and actually increases inhibitory

receptor expression in these cells. Unlike memory cells, exhausted CD8$^+$ T cells do not require the homeostatic cytokines IL-7 and IL-15 to persist and instead rely on constant exposure to their cognate antigen (107). Thus, miR-155's ability to alter CD8$^+$ T cell sensitivity to common gamma chain cytokine receptor signals is unlikely to be responsible for increased persistence of miR-155-overexpressing exhausted T cells. Conversely, in the chronic setting of cancer, miR-155 overexpression delays CD8$^+$ T cell contraction, prolongs cytokine production, and increases their sensitivity to common gamma chain cytokines (44). miR-31 may reduce exhaustion in part by increasing CD8$^+$ T cell sensitivity to type I IFNs (105). However, miR-155 decreases IFN sensitivity (29), so this is also unlikely to play a role in the persistence of miR-155 overexpressing exhausted T cells. The varied effects of miR-155 on T cells responses to acute and chronic infection as well as cancer highlight the context specific nature of miRNAs regulation of immunity.

Perspective

miRNAs are important regulators of CD8$^+$ T cell function in host defense, infection, and cancer immunosurveillance. They regulate almost every aspect of CD8$^+$ T cell behavior from survival and proliferation, to the acquisition and deployment of effector functions, to fate decisions that dictate the formation of immunological memory and tolerance. Individual miRNA-target interactions can have profound impacts on cell behavior, but mounting evidence indicates that miRNAs mediate context-specific biological effects by binding and tuning the expression of large networks of target genes. There remains much to learn about miRNA regulation in CD8$^+$ T cells, and perhaps even more to learn about CD8$^+$ T cell programming from the study of miRNAs. While somewhat useful in the validation of target sites, assays that remove the 3'UTRs of putative target genes from their endogenous context (e.g., luciferase assays) suffer from a potential for both false-positive and false-negative findings. Furthermore, the singling out of individual targets and the highlighting of the capacity for their 3'UTRs to be regulated by a particular miRNA draws attention away from the underlying nature of miRNAs as network regulators of gene circuits. The field of miRNA biology will benefit greatly from a more systematic identification of miRNA targets within each cellular context and formulating a catalog of the pathways and gene sets involved. To this end, future studies should expand on efforts to map the full target repertoire of functionally relevant miRNAs using a combination of bioinformatics, biochemistry (AHC), and analysis of endogenous miRNA effects on gene expression in biologically relevant contexts. These target-agnostic approaches invite the discovery of novel targets and will lead to better assessment of how their associated pathways integrate with phenotype.

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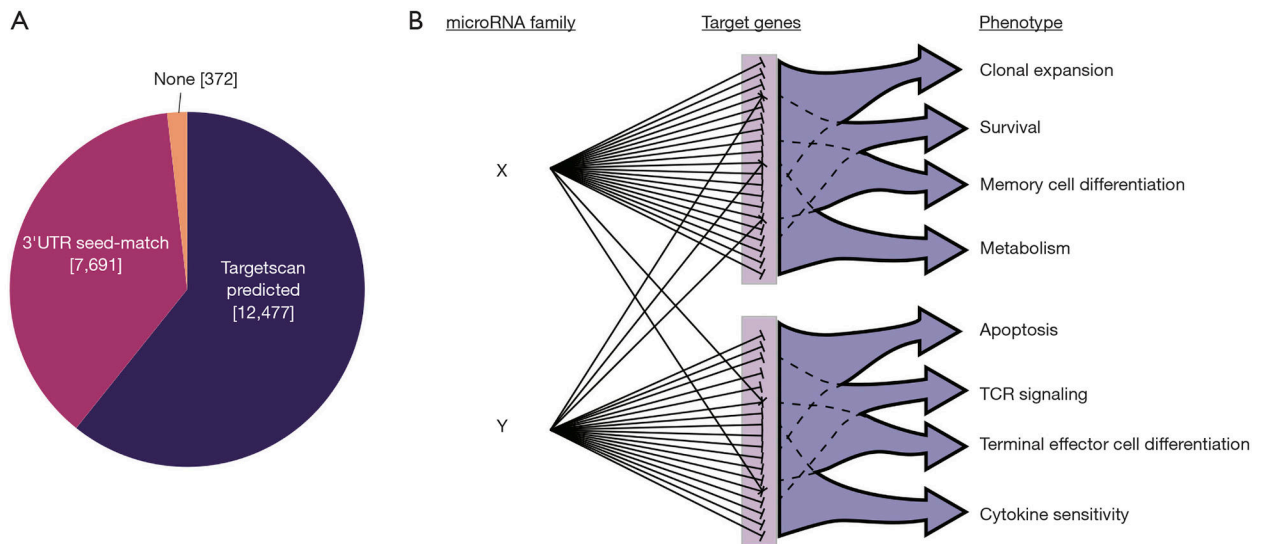
Figure 1.
MicroRNAs (miRNAs) target broad, overlapping networks of target genes to elicit phenotypes. (A) Characterization of RefSeq annotated genes with the potential for targeting by miRNAs based on TargetScan prediction algorithm or 3'UTR seed-match. (B) Schematic of how individual miRNAs can regulate networks of genes shared between pathways and individual mRNAs can be regulated by multiple miRNAs.