Review

Regulation of Memory CD8⁺ T Cell Differentiation by MicroRNAs

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Key Words

MicroRNAs • Memory T cells • Adoptive T cell therapy

Abstract

MicroRNAs (miRNAs) have emerged as crucial regulators of T lymphocyte survival, differentiation and function, all of which are key factors impacting the outcome of adoptive T cell-based immunotherapy. It has become increasingly clear that the adoptive transfer of memory CD8⁺ T cell subsets is highly correlated with objective clinical responses for patients with advanced cancer. However, it is unclear how to improve the long-term persistence of transferred CD8⁺ T cells using miRNAs. Here, we highlight the current advances in our understanding of the role of miRNAs in regulating the differentiation of memory CD8⁺ T cells. We specifically discuss the effect of miRNAs on key transcription factors, immune checkpoints and signal pathways, which contribute to the differentiation of effector and memory T cell subsets. Ultimately, miRNAs may be easily integrated into existing T cell receptor (TCR) and chimeric antigen receptor (CAR) platforms to promote adoptive T cell therapy with multiple advantages. Thus, combining T cell-based therapy with miRNAs could be considered a promising and robust strategy for cancer treatment.

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Introduction

Memory CD8⁺ T cells play a critical role in the immune response against pathogens and cancer [1]. After stimulation with foreign antigens, naïve T (T_n) cells are activated and differentiate into effector T (T_{eff}) cells and acquire effector functions, including the production of effector cytokines and the expression of a large number of effector genes [2]. After the peak of T cell proliferation, most T_{eff} cells die via apoptosis. The surviving T cells differentiate into memory T cells, including stem cell memory T (T_{scm}) cells, central memory T (T_{cm}) cells, and effector memory T (T_{em}) cells [3]. Memory T cells display robust

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proliferative and survival advantages, and different subsets of memory T cells can be identified by specific cell surface markers and gene expression profiles [4]. Following adoptive transfer, naturally occurring or gene engineered Teff cells enter the tumor microenvironment, where they are regulated by a complex immunosuppressive network that consists of cancer cells, inflammatory cells, stromal cells and cytokines [5]. These components often drive T cells to undergo terminal differentiation, in which they express high levels of co-inhibitory receptors, including programmed cell death protein 1 (PD-1), T cell immunoglobulin domain and mucin domain protein 3 (TIM-3), and cytotoxic T lymphocyte antigen-4 (CTLA-4) [6, 7]. In addition, current *in vitro* methods to expand T cells to sufficient numbers for adoptive transfer cause terminal differentiation and replicative senescence of the adoptively transferred cells [8]. Thus, efforts to delineate the mechanisms driving T cell expansion and differentiation in CD8⁺ T cells may improve the efficacy of adoptive T cell therapy (ACT).

MicroRNAs (miRNAs) are endogenous single-stranded RNA molecules of 18–24 nucleotides in length that post-transcriptionally inhibit the expression of their target mRNAs in cells by binding to their 3' untranslated region (UTR) [9, 10]. It has been predicted that up to 90% of human protein-coding genes are regulated by miRNAs. Furthermore, a single protein can be influenced by several individual miRNAs targeting shared or distinct sequences within the target 3' UTR. Therefore, miRNAs can potentially regulate biological networks by cumulatively or cooperatively inhibiting molecules [11–13]. miRNAs that mediate the regulation of gene and protein expression have emerged as an important mechanism in T cell biological processes [14–16]. It has become clear that transcription factors and metabolic signaling pathways can contribute to T cell differentiation and memory formation [17–19]. Recent studies have also highlighted the importance of cytokines in enhancing the formation of Tscm cells for ACT [20–22]. Mounting evidence demonstrates that miRNAs are critical regulators of the transcription factors, immune checkpoints and pathways involved in the regulation of memory T cell differentiation [23, 24]. Here, we review the mechanisms by which miRNAs regulate the differentiation of memory CD8⁺ T cells and highlight potential strategies to integrate miRNAs into ACT for the therapeutic benefit of patients with advanced cancer.

Biological characteristics of miRNAs in CD8⁺ T cells

During miRNA biogenesis, nascent primary miRNA transcripts are sequentially processed in the nucleus by the type III ribonuclease called drosha and the non-catalytic protein DGCR8 into stem-loop structures called precursor miRNAs [25]. These precursor miRNAs are then transported by exportin-5 into the cytoplasm, and are further processed into the mature form of miRNA by the RNase III endonuclease Dicer [26]. The regulation of T cell fate decision and control of development and differentiation in thymocytes by mature miRNAs have been reported [27]. A deficiency of Dicer in T cells leads to the dysregulation of CD8⁺ T cell activation, proliferation and survival, indicating that miRNAs are major gene regulators during CD8⁺ T cell responses [28]. Therefore, the properties of miRNAs may be particularly relevant for the long-term survival of adoptively transferred T cells [29]. Extensive studies have indeed provided a comprehensive view of the expression and functionality of several

Table 1. Regulation of CD8⁺ T cell differentiation by miRNAs

miRNA	Target gene	Cytokine(s) modulated	Function
miR-15b	DEDD	IFN- γ ↓	Inhibit the apoptosis of CD8 ⁺ T cells and induce T cells to a memory state [54].
miR17-92	PTEN	IFN- γ ↑	Promote the proliferation and terminal differentiation of CD8 ⁺ T cells [36, 37].
miR-28	PDCD1 HAVCR2 BTLA	IL-2 ↑ TNF- α ↑	Reduce exhausted T cells [46].
miR-139/miR-342	EOMES	Perforin ↓	Control effector CD8 ⁺ T cell differentiation at multiple levels [28].
miR-150	TGFBR1, TGFBR2 SOCS1	IFN- γ IL-17A ↓	Promote memory cell generation [53].
miR-155	SHIP-1	IFN- γ ↑ TNF- α ↑	Enhance the effector function and promote the terminal differentiation of CD8 ⁺ T cells [50].

specific miRNAs in different subsets of CD8⁺ T cells [30, 31]. The differentiation of CD8⁺ T cells must be tightly regulated to induce productive immune responses and to establish long-term immunological memory. Hence, we will review individual miRNAs that are specifically expressed in mature T lymphocytes, with a particular focus on those influencing CD8⁺ T cell differentiation and function. The key miRNAs discussed in this review and the processes in which they are involved are summarized in Table 1.

Important miRNAs for the differentiation of memory CD8⁺ T cells

Systematic miRNA profiling studies have shown that the expression of multiple miRNAs changes during T cell differentiation, suggesting that miRNAs are likely involved in this process [27]. With the development of next generation sequencing technology, the view of the miRNA landscape in CD8⁺ T cells has become increasingly clear. However, the key miRNAs that may determine the differentiation status of CD8⁺ T cells remain to be defined. Here, we describe how the differentiation and effector function of T cells are regulated by several miRNAs.

miR-17-92

The miR-17-92 cluster, a well-studied miRNA cluster, is a polycistronic gene encoding 6 miRNAs (miR-17, miR-18a, miR-19a, miR-19b, miR-20a and miR-92) and has 2 paralogs (miR-106b-25 and miR-106a-363) generated by ancient genomic duplications [32, 33]. miR-17-92 is frequently relevant in genomic translocation and amplification, and is overexpressed in various malignancies [34, 35]. This miRNA cluster is expressed at a high level in T precursor cells, and is decreased after clonal expansion. Deletion of miR-17-92 results in enhanced memory differentiation in mice. In contrast, the increased expression of miR-17-92 leads to the skewed terminal effector differentiation of CD8⁺ T cells, with alterations in a series of transcription factors that balance memory and effector fates. Therefore, miR-17-92 down-regulation is required for normal memory formation [36]. Mechanistically, miR-17-92 regulates CD8⁺ T cell proliferation and phosphatase and tensin homolog (PTEN)-mediated regulation of the PI3K-Akt-mammalian target of rapamycin (mTOR) axis [37]. mTOR is an important mediator of programmed CD8⁺ effector and memory cell fate by regulating the expression of T-box transcription factor (TBX21) and eomesodermin (EOMES) [38, 39]. Further evidence supports a role for miR-17-92 in targeting HIF-1 α in cancer cells. HIF-1 α is also expressed in CD8⁺ T cells and may stimulate glycolysis and enhance effector function [40, 41]. Consistently, miR-17-92 overexpression has been reported to promote the development of terminal effectors and decrease the formation of memory T cells by enhancing mTOR activity [36].

miR-28

Most T cells in the tumor microenvironment are exhausted, leading to tumor immune evasion [42, 43]. T cell exhaustion may be related to defective memory T cell formation, and dysfunctional T cells are ineffective at eliminating tumor cells. The major feature of the exhausted T cell phenotype is the very high expression of immune checkpoint molecules such as PD-1 [44]. Immune checkpoint inhibitors targeting PD-1/programmed death-ligand 1 (PD-L1) have been clinically successful and have revolutionized the treatment of advanced cancers [45]. Furthermore, research has been performed to observe the effects of miRNAs on PD-1 in cancer and T cell exhaustion [46]. On the basis of the gene silencing function of miRNAs, miR-28 has emerged as a potential target for cancer therapy. miR-28 is an intragenic miRNA encoded by intron 6 of the LIM domain-containing preferred translocation partner in lipoma gene located on human chromosome 3q28 [47]. It has been reported that expression of miR-28 is down-regulated by approximately 30% in exhausted PD-1⁺ T cells extracted from melanomas [46]. miR-28 inhibits the expression of the immune checkpoint molecules PD-1, TIM3 and BTLA in T cells by binding to their respective 3' UTRs. Experimentally, the

addition of miR-28 mimics can convert the exhausted phenotype of PD-1⁺ T cells, at least in part, by restoring the secretion of interleukin-2 (IL-2) and tumor necrosis factor (TNF)- α in T cells in the tumor microenvironment [46]. Collectively, miR-28 may play an important role in reversing the terminal status of T cells into memory cells and recovering the ability of T cells to secrete pro-inflammatory cytokines.

miR-155

The B-cell integration cluster, which encodes miR-155, was initially found to accumulate in human B cell lymphoma patients [48]. The differential expression of miR-155 is involved in the differentiation of CD8⁺ T cells. For example, miR-155 expression is up-regulated in effector and effector memory CD8⁺ T cells, while it is down-regulated in Tn and Tcm cells [49]. It appears that T cell receptor (TCR) signaling and the NF- κ B pathway are involved in the induction of miR-155 expression in CD8⁺ T cells, though the underlying factors regulating its expression are multifaceted. miR-155 deficiency results in defective PI3K-AKT signaling, due to heightened levels of the inositol 5-phosphatase SHIP-1, a known target of miR-155. miR-155 deficiency also causes defective cytokine signaling through STAT5 by targeting cytokine signaling 1 (SOCS-1) and the protein tyrosine phosphatase PTPN2 [49]. Therefore, miR-155 is required for the effector response of CD8⁺ T cells. More importantly, a lack of miR-155 causes a skewing of CD8⁺ T cells toward the development of memory precursor cells [50]. Although central memory CD8⁺ T cells have been shown to confer superior immunity both *in vivo* and in studies using adoptive cell transfer [3], it remains elusive whether manipulation of miR-155 expression in T cells may prove a useful way to generate preferentially memory cells for immunotherapy.

Other miRNAs

Recent studies on miRNA profiling have identified dynamic changes in the miRNA repertoire of Tn cells as they differentiate into functional effector and memory cells [51]. The striking global down-regulation of a number of miRNAs, including miR-16, miR-21, miR-142, miR-150, miR-15b and let-7f, was observed in effector CD8⁺ T cells compared with Tn cells. However, the reduced expression of these miRNAs tends to increase their expression to levels similar to those in Tn cells [52]. miR-150 represses the expression of the IL-2 receptor alpha chain (IL-2R α , also called CD25) in CD8⁺ T cells. In line with these results, miR-150^{-/-} T cells express higher levels of IL-2R α , undergo more proliferation, and rapidly become terminally differentiated. Therefore, miR-150 is likely responsible for the survival of effector CD8⁺ T cells and their differentiation into memory cells by attenuating IL-2 signaling [53]. In addition, miR-15b was reported to inhibit the activation of CD8⁺ T cells by repressing the production of IL-2, interferon (IFN)- γ , and CD69 and induces T cells to develop into a memory state [54]. Moreover, miR-15b prevents CD8⁺ T cells from undergoing apoptosis by targeting DEDD, a ubiquitous death effector domain-containing protein [54]. miR-29 regulates the production of IFN- γ by T cells by binding directly to the 3' UTR of IFN- γ mRNA, as well as TBX21 and EOMES [55]. During the *in vivo* immune response to lymphocytic choriomeningitis virus (LCMV), the number of TBX21-dependent short-lived effector cells (SLECs) is significantly decreased by overexpression of miR-29 [56]. The activation of T cells via TCR signaling induces a high level of miR-31 expression, which promotes T cell dysfunction by enhancing the expression of multiple inhibitory molecules. In addition, miR-31 has an important role in enhancing the exhaustion of CD8⁺ T cells [57]. Two other miRNAs, miR-139 and miR-342, which are down-regulated in Tef cells compared with memory precursor T cells in LCMV-infected mice, are involved in the terminal effector cell differentiation of CD8⁺ T cells [28]. In light of the important roles of miRNAs in T cell differentiation and function, decreasing or increasing the levels of miRNAs in T cells could be a promising strategy for future immunotherapeutic approaches.

miRNA-mediated regulation of transcription factors, immune checkpoints and signaling pathways during the differentiation of memory CD8⁺ T cells

The target networks of miRNAs are complex and include many molecules and signaling pathways [58]. Indeed, the role of miRNAs in the differentiation of various T cell subsets is mediated by different molecules such as transcription factors and immune checkpoints, resulting in memory or terminal formation of CD8⁺ T cells (Fig. 1). The following sections provide insights into the diverse roles of miRNAs as key regulators of these molecules and related pathways.

miRNA regulation of transcription factors

It is well known that the development of memory CD8⁺ T cells is crucially dependent on transcriptional events that are regulated by TBX21 and EOMES [59]. Furthermore, the high expression of TBX21 is associated with the terminal differentiation of SLECs. Conversely, the expression of EOMES is associated with a less differentiated phenotype of central memory T cells [60, 61]. Over the past decade, several miRNAs have been found to regulate the expression of EOMES and TBX21 in CD8⁺ T cells [62]. To explore further the effects of miRNAs on the expression of these transcription factors, miRNA knockout in T cells has been studied extensively. For example, miR-17-92 deficiency in CD8⁺ T cells reduces the expression of EOMES, but increases the expression of TBX21, leading to the formation of CD127^{lo}KLRG-1^{hi} SLECs [63]. Additionally, miR-139 and miR-342 target and inhibit the expression of EOMES in effector cells [28], which is in keeping with the hypothesis that EOMES is a contributor to the formation of memory precursor cells [59]. These findings have indicated an important role for miRNAs in the differentiation of memory T cells by regulating the expression of transcription factors.

miRNA regulation of immune checkpoints

Tumor-infiltrating CD8⁺ T cells can mount responses against many human tumor types. However, these CD8⁺ T cell responses often fail to eradicate tumors because T cells can become exhausted, in which state they express high levels of PD-1, TIM-3, and BTLA [64]. Moreover, the upregulation of those inhibitory receptors correlates with variable levels of T cell dysfunction [7]. It is worth mentioning that the PD-L1/PD-1 signaling pathway is a crucial regulator of T cell exhaustion in cancer [65]. Despite the fact that PD-1 blockade monotherapy can partially reinvigorate exhausted T cells, only a few studies have attempted to examine the role of miRNAs in regulating the PD-1 pathway. To explore the effects of miRNAs on immune checkpoints, a microarray was used to profile differentially expressed miRNAs between PD-1⁺ and PD-1⁻ T cells. Several miRNAs, including miR-28, miR-150, and

Fig. 1. CD8⁺ T cell differentiation is regulated by miRNAs. miR-17-92 targets PTEN to promote the activation of the PI3K-mTOR signaling pathway. mTOR is an important mediator of the generation of CD8⁺ effector T cells through TBX21 up-regulation and EOMES down-regulation. The NF-κB signaling pathway induces the expression of miR-155, which further augments IFN-γ production by targeting SHIP1. miR-155 also promotes the differentiation of effector T cells by activating mTOR. miR-139 and miR-342 can target EOMES directly, thereby inhibiting the generation of memory T cells. In addition, miR-28 and miR-138 can target immune checkpoints such as PD-1 and TIM-3. Blocking the PD-1 pathway may reverse the function of effector T cells and promote the generation of memory T cells.

miR-151-5p, are significantly down-regulated in PD-1⁺ T cells from mice with melanoma. Eventually, miR-28 was identified as a key miRNA that can directly target the 3' UTR of PD-1, which may convert the exhausted status of T cells by inducing the secretion of cytokines such as IL-2 and TNF- α [46]. Other miRNAs such as miR-138 were also found to target PD-1 by binding to its 3' UTR [66]. Thus, an attractive option for reversing exhausted T cells is to block inhibitory receptors such as PD-1 by manipulating miRNAs. PD-L1 is expressed abundantly in tumor cells and stromal cells such as myeloid-derived suppressor cells and macrophages [67]. Recent studies demonstrated that PD-L1 expression can be inhibited by specific miRNAs expressed in cancer cells [68]. For example, miR-34a and miR-138-5p inhibit PD-L1 expression directly by binding to its 3' UTR [69]. Blocking the PD-1/PD-L1 pathway by the manipulation of miRNAs may provide a potential strategy to reinvigorate the effector function of exhausted CD8⁺ T cells. Given that the generation of long-lived memory T cells is a goal of effective immunotherapy, a more detailed understanding of immune checkpoint-mediated tumor evasion and how miRNAs might be manipulated to target these immune checkpoints, warrants further investigation.

miRNA regulation of signaling pathways

From a signaling perspective, central roles for the evolutionarily conserved PI3K family member mTOR in regulating T cell differentiation have emerged [70]. Indeed, an inhibitor of mTOR promotes the formation of memory T cells, providing an effective strategy for ACT [71, 72]. Additionally, the activation of mTOR induces the expression of the glucose transporter Glut1, which is required for glucose uptake by Teff cells [38]. In this regard, altering the metabolic program via the mTOR pathway provides an integrative function for miRNAs in regulating T cell differentiation. The expression of miR-17-92 and miR-155 is positively correlated with the activation status of the mTOR complex [27], thereby enhancing the effector function and glycolysis of CD8⁺ T cells. Meanwhile, the formation of memory CD8⁺ T cells can be suppressed by these two miRNAs, while oxidative phosphorylation, an important metabolic pathway of CD8⁺ T cells, was diminished at the same time. Whether miRNAs can regulate the metabolism of CD8⁺ T cells by targeting metabolic pathways remains to be determined.

Growing evidence indicates that miRNAs are also involved in regulating the Notch and Wnt/ β -catenin signaling pathways, which play an important role in the differentiation of memory T cells [73, 74]. A recent study indicated that Notch signaling redirects differentiation from memory precursor-like activated CD8⁺ T cells to stem cell memory T-like cells, which have a long-lived and self-renewing potential [75]. Interestingly, miRNAs, such as miR-34, miR-598 and miR-433, regulate cancer stem cells by targeting the molecules of the Notch signaling pathway [76-78]. However, the role of these miRNAs in the differentiation of T cells via the Notch pathway is still largely undefined. miR-150 has an effect on the repression of Notch3, thereby inhibiting the differentiation of T cells [79]. Thus, miRNAs could regulate CD8⁺ T cell differentiation by targeting the Notch signaling pathway.

Integrating miRNAs into the design of future immunotherapeutic approaches

The emergence of engineered T cells as a type of cancer therapy marks the beginning of a new era in medicine, providing an innovative way to combat cancer. The history of TCR-engineered T cells or CAR T cells has shown the absolute clinical importance of T cell expansion and persistence in patients with cancer [80, 81]. Within the past few years, many clinical trials have indicated that CD19 CAR-T cells can induce durable remission as well as prolong disease-free survival of patients with refractory B cell malignancies [82]. Furthermore, the success of CD19 CAR-T cell treatment depends on its functionality and persistence. However, current T cell therapies had a limited clinical response in solid tumors [83]. Therefore, there are emerging challenges in the field of T cell-based immunotherapy for solid tumors: (1) how can we expand less-differentiated engineered T cell subsets *in vitro*; and (2) is it necessary

to integrate different therapeutic strategies into a more powerful cell therapy [84].

Accumulating findings from *in vitro* and *in vivo* models support the critical role of miRNAs in regulating the development, survival, differentiation and effector functions of CD8⁺ T cells [85]. A broader understanding of the miRNAs that control long-lived and durable T cell function is needed for the improvement of T cell-based immunotherapy.

Nevertheless, whether a combination of miRNAs and engineered T cells could improve the efficacy of T cell therapy remains elusive. At present, it was found that miR-153 inhibits IDO1 expression in cancer cells, and which is a tumor-suppressive miRNA that enhances CAR-T cell immunotherapy for solid tumors [86]. More importantly, the over-expression of the miR-17-92 cluster in CAR-transduced T cells results in prolonged protection against tumor re-challenge in a xenograft model of glioma, indicating the potential of engineered T cells with miRNAs for the treatment of cancer [87]. There are also studies demonstrating better antitumor responses and long-term persistence when both CD4⁺ and CD8⁺ CAR-T cells are transferred [88], and it has been proven that one critical role for miRNAs in CD4⁺ T cells is to help maintain the functional phenotypes of distinct T cell lineages [58]. As shown in Fig. 2, T cells isolated from patients with cancer can be genetically engineered to recognize tumor-associated antigens in various ways. Recombinant retroviruses can be used particularly to transduce T cells with the TCR or CAR, which generates antigen specificity or antibody-like specificity. To improve the survival of TCR- and CAR-T cells, retroviruses encoding miRNAs may be further transduced to T cells and used for immunotherapy. Although the genetic modification of tumor-specific T cells with miRNAs holds great promise, it must be approached with caution to ensure safety when introducing genes or miRNAs. More importantly, the effects of miRNAs on the effector function and differentiation of CD4⁺ T cells need more exploration when considering their adoptive transfer to patients with cancer.

Fig. 2. Integrating different therapeutic strategies for T cell-based immunotherapy. T cells can be genetically engineered to recognize tumor-associated antigens in a variety of ways. T cells isolated from a patient with cancer can be modified with retroviral vectors encoding the desired TCR sequences or CARs. Other retroviral vectors that encode miRNAs can be further transduced into these modified T cells, resulting in the promotion of memory TCR- or CAR-T cell generation. These miRNA-modified memory TCR- or CAR-T cells are subsequently re-infused back into the patient for adoptive cellular therapy.

Perspectives

miRNAs and their target genes have an emerging role in regulating T cell differentiation and show great potential for T cell-based cancer immunotherapy. miRNAs are involved in many aspects of T cell biology, including transcription factors, immune checkpoints and signaling pathways, which can have a direct impact on the differentiation and functionality of CD8⁺ T cells. With the development of next generation sequencing technology, a fascinating world has opened up for more detailed investigations of the fine-tuning of the regulation of T cell differentiation by miRNAs.

An ever-growing understanding of miRNA-mediated regulation networks will not only continue to reveal the beauty of nature in CD8⁺ T cell differentiation but also bring us closer to the prospect of safer and more effective T cell therapies in combination with miRNA-based therapeutics. Harnessing miRNAs in TCR- and CAR-T cells has multiple advantages over other gene engineering strategies. Due to their unique ability to target multiple genes simultaneously, the manipulation of a single miRNA may radically change T cell differentiation and function. Meanwhile, owing to their small size, miRNA genes can be integrated easily

into existing multicistronic TCR and CAR platforms. In summary, miRNA gene engineering of tumor-specific T cells is a powerful and flexible approach that holds great potential to revolutionize next-generation T cell-based immunotherapies.

Abbreviations

Tn; (naive T cells); Teff (effector T cells); Tscm (stem cell memory T cells); Tcm (central memory T cells); Tem (effector memory T cells); PD-1 (programmed cell death protein 1); TIM-3 (T cell immunoglobulin domain and mucin domain protein 3); CTLA-4 (cytotoxic T lymphocyte antigen-4); ACT (adoptive T cell therapy); UTR (untranslated region); PTEN (phosphatase and tensin homolog); mTOR (mammalian target of rapamycin); TBX21 (T-box transcription factor); EOMES (eomesodermin); IL-2 (interleukin-2); TNF (tumor necrosis factor); IFN (interferon); SLECs (short-lived effector cells); TCR (T cell receptor); PD-L1 (programmed death-ligand 1); CAR (chimeric antigen receptor).

Disclosure Statement

The authors declare there is no conflict of interests.

References

- Gattinoni L, Restifo NP: Moving T memory stem cells to the clinic. *Blood* 2013;121:567-568.
- Pulko V, Davies JS, Martinez C, Lanteri MC, Busch MP, Diamond MS, Knox K, Bush EC, Sims PA, Sinari S, Billheimer D, Haddad EK, Murray KO, Wertheimer AM, Nikolich-Zugich J: Human memory T cells with a naive phenotype accumulate with aging and respond to persistent viruses. *Nat Immunol* 2016;17:966-975.
- Klebanoff CA, Gattinoni L, Restifo NP: Sorting through subsets: which T-cell populations mediate highly effective adoptive immunotherapy? *J Immunother* 2012;35:651-660.
- Chang JT, Wherry EJ, Goldrath AW: Molecular regulation of effector and memory T cell differentiation. *Nat Immunol* 2014;15:1104-1115.
- Wu AA, Drake V, Huang HS, Chiu S, Zheng L: Reprogramming the tumor microenvironment: tumor-induced immunosuppressive factors paralyze T cells. *Oncoimmunology* 2015;4:e1016700.
- Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, Polley A, Betts MR, Freeman GJ, Vignali DA, Wherry EJ: Coregulation of CD8⁺ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol* 2009;10:29-37.
- Fourcade J, Sun Z, Pagliano O, Guillaume P, Luescher IF, Sander C, Kirkwood JM, Olive D, Kuchroo V, Zarour HM: CD8(+) T cells specific for tumor antigens can be rendered dysfunctional by the tumor microenvironment through upregulation of the inhibitory receptors BTLA and PD-1 *Cancer Res* 2012;72:887-896.
- Restifo NP, Smyth MJ, Snyder A: Acquired resistance to immunotherapy and future challenges. *Nat Rev Cancer* 2016;16:121-126.
- Ambros V, Bartel B, Bartel DP, Burge CB, Carrington JC, Chen X, Dreyfuss G, Eddy SR, Griffiths-Jones S, Marshall M, Matzke M, Ruvkun G, Tuschl T: A uniform system for microRNA annotation. *Rna* 2003;9:277-279.
- Xiao C, Rajewsky K: MicroRNA control in the immune system: basic principles. *Cell* 2009;136:26-36.
- Krek A, Grun D, Poy MN, Wolf R, Rosenberg L, Epstein EJ, MacMenamin P, da Piedade I, Gunsalus KC, Stoffel M, Rajewsky N: Combinatorial microRNA target predictions. *Nat Genet* 2005;37:495-500.
- Selbach M, Schwanhauss B, Thierfelder N, Fang Z, Khanin R, Rajewsky N: Widespread changes in protein synthesis induced by microRNAs. *Nature* 2008;455:58-63.
- Cui Q, Yu Z, Purisima EO, Wang E: Principles of microRNA regulation of a human cellular signaling network. *Mol Syst Biol* 2006;2:46.

- 14 O'Connell RM, Rao DS, Chaudhuri AA, Baltimore D: Physiological and pathological roles for microRNAs in the immune system. *Nat Rev Immunol* 2010;10:111-122.
- 15 Jeker LT, Bluestone JA: MicroRNA regulation of T-cell differentiation and function. *Immunol Rev* 2013;253:65-81.
- 16 Aalaei-Andabili SH, Rezaei N: MicroRNAs (MiRs) Precisely Regulate Immune System Development and Function in Immunosenescence Process. *Int Rev Immunol* 2016;35:57-66.
- 17 Kallies A: Distinct regulation of effector and memory T-cell differentiation. *Immunol Cell Biol* 2008;86:325-332.
- 18 Yu B, Zhang K, Milner JJ, Toma C, Chen R, Scott-Browne JP, Pereira RM, Crotty S, Chang JT, Pipkin ME, Wang W, Goldrath AW: Epigenetic landscapes reveal transcription factors that regulate CD8⁺ T cell differentiation. *Nat Immunol* 2017;18:573-582.
- 19 Siska PJ, Rathmell JC: T cell metabolic fitness in antitumor immunity. *Trends Immunol* 2015;36:257-264.
- 20 Rubinstein MP, Lind NA, Purton JF, Filippou P, Best JA, McGhee PA, Surh CD, Goldrath AW: IL-7 and IL-15 differentially regulate CD8⁺ T-cell subsets during contraction of the immune response. *Blood* 2008;112:3704-3712.
- 21 Pilipow K, Roberto A, Roederer M, Waldmann TA, Mavilio D, Lugli E: IL15 and T-cell Stemness in T-cell-Based Cancer Immunotherapy. *Cancer Res* 2015;75:5187-5193.
- 22 Sabatino M, Hu J, Sommariva M, Gautam S, Fellowes V, Hocker JD, Dougherty S, Qin H, Klebanoff CA, Fry TJ, Gress RE, Kochenderfer JN, Stronck DF, Ji Y, Gattinoni L: Generation of clinical-grade CD19-specific CAR-modified CD8⁺ memory stem cells for the treatment of human B-cell malignancies. *Blood* 2016;128:519-528.
- 23 Bartel DP: MicroRNAs: target recognition and regulatory functions. *Cell* 2009;136:215-233.
- 24 Smolle MA, Calin HN, Pichler M, Calin GA: Noncoding RNAs and immune checkpoints-clinical implications as cancer therapeutics. *Febs j* 2017;10.1111/febs.14030
- 25 Wang Y, Medvid R, Melton C, Jaenisch R, Billewicz R: DGC8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal. *Nat Genet* 2007;39:380-385.
- 26 Haftmann C, Stittrich AB, Sgouroudis E, Matz M, Chang HD, Radbruch A, Mashreghi MF: Lymphocyte signaling: regulation of FoxO transcription factors by microRNAs. *Ann N Y Acad Sci* 2012;1247:46-55.
- 27 Saki N, Abroun S, Soleimani M, Hajizamani S, Shahjehani M, Kast RE, Mortazavi Y: Involvement of MicroRNA in T-Cell Differentiation and Malignancy. *Int J Hematol Oncol Stem Cell Res* 2015;9:33-49.
- 28 Trifari S, Pipkin ME, Bandukwala HS, Aijo T, Bassein J, Chen R, Martinez GJ, Rao A: MicroRNA-directed program of cytotoxic CD8⁺ T-cell differentiation. *Proc Natl Acad Sci U S A* 2013;110:18608-18613.
- 29 Ji Y, Hocker JD, Gattinoni L: Enhancing adoptive T cell immunotherapy with microRNA therapeutics. *Semin Immunol* 2016;28:45-53.
- 30 Almanza G, Fernandez A, Volinia S, Cortez-Gonzalez X, Croce CM, Zanetti M: Selected microRNAs define cell fate determination of murine central memory CD8 T cells. *PLoS One* 2010;5:e11243.
- 31 Sheppard HM, Verdon D, Brooks AE, Feisst V, Ho YY, Lorenz N, Fan V, Birch NP, Didsbury A, Dunbar PR: MicroRNA regulation in human CD8⁺ T cell subsets--cytokine exposure alone drives miR-146a expression. *J Transl Med* 2014;12:292.
- 32 Chakraborty S, Mehtab S, Patwardhan A, Krishnan Y: Pri-miR-17-92a transcript folds into a tertiary structure and autoregulates its processing. *Rna* 2012;18:1014-1028.
- 33 Du P, Wang L, Sliz P, Gregory RI: A Biogenesis Step Upstream of Microprocessor Controls miR-17 approximately 92 Expression. *Cell* 2015;162:885-899.
- 34 Quattrochi B, Gulvady A, Driscoll DR, Sano M, Klimstra DS, Turner CE, Lewis BC: MicroRNAs of the mir-17~92 cluster regulate multiple aspects of pancreatic tumor development and progression. *Oncotarget* 2017;10.18632/oncotarget.16277
- 35 Li H, Wu Q, Li T, Liu C, Xue L, Ding J, Shi Y, Fan D: The miR-17-92 cluster as a potential biomarker for the early diagnosis of gastric cancer: evidence and literature review. *Oncotarget* 2017;10.18632/oncotarget.15023
- 36 Wu T, Wieland A, Araki K, Davis CW, Ye L, Hale JS, Ahmed R: Temporal expression of microRNA cluster miR-17-92 regulates effector and memory CD8⁺ T-cell differentiation. *Proc Natl Acad Sci U S A* 2012;109:9965-9970.
- 37 Khan AA, Penny LA, Yuzefpolskiy Y, Sarkar S, Kalia V: MicroRNA-17~92 regulates effector and memory CD8 T-cell fates by modulating proliferation in response to infections. *Blood* 2013;121:4473-4483.

- 38 Pollizzi KN, Powell JD: Integrating canonical and metabolic signalling programmes in the regulation of T cell responses. *Nat Rev Immunol* 2014;14:435-446.
- 39 Rao RR, Li Q, Odunsi K, Shrikant PA: The mTOR kinase determines effector versus memory CD8⁺ T cell fate by regulating the expression of transcription factors T-bet and Eomesodermin. *Immunity* 2010;32:67-78.
- 40 Taguchi A, Yanagisawa K, Tanaka M, Cao K, Matsuyama Y, Goto H, Takahashi T: Identification of hypoxia-inducible factor-1 alpha as a novel target for miR-17-92 microRNA cluster. *Cancer Res* 2008;68:5540-5545.
- 41 Fernandez-Ramos AA, Poindessous V, Marchetti-Laurent C, Pallet N, Lorient MA: The effect of immunosuppressive molecules on T-cell metabolic reprogramming. *Biochimie* 2016;127:23-36.
- 42 Wherry EJ: T cell exhaustion. *Nat Immunol* 2011;12:492-499.
- 43 Jiang Y, Li Y, Zhu B: T-cell exhaustion in the tumor microenvironment. *Cell Death Dis* 2015;6:e1792.
- 44 Sen DR, Kaminski J, Barnitz RA, Kurachi M, Gerdemann U, Yates KB, Tsao HW, Godec J, LaFleur MW, Brown FD, Tonnerre P, Chung RT, Tully DC, Allen TM, Frahm N, Lauer GM, Wherry EJ, Yosef N, Haining WN: The epigenetic landscape of T cell exhaustion. *Science* 2016;354:1165-1169.
- 45 Mahoney KM, Freeman GJ, McDermott DF: The Next Immune-Checkpoint Inhibitors: PD-1/PD-L1 Blockade in Melanoma. *Clin Ther* 2015;37:764-782.
- 46 Li Q, Johnston N, Zheng X, Wang H, Zhang X, Gao D, Min W: miR-28 modulates exhaustive differentiation of T cells through silencing programmed cell death-1 and regulating cytokine secretion. *Oncotarget* 2016;7:53735-53750.
- 47 Schneider C, Setty M, Holmes AB, Maute RL, Leslie CS, Mussolin L, Rosolen A, Dalla-Favera R, Basso K: MicroRNA 28 controls cell proliferation and is down-regulated in B-cell lymphomas. *Proc Natl Acad Sci U S A* 2014;111:8185-8190.
- 48 Vigorito E, Perks KL, Abreu-Goodger C, Bunting S, Xiang Z, Kohlhaas S, Das PP, Miska EA, Rodriguez A, Bradley A, Smith KG, Rada C, Enright AJ, Toellner KM, MacLennan IC, Turner M: microRNA-155 regulates the generation of immunoglobulin class-switched plasma cells. *Immunity* 2007;27:847-859.
- 49 Dudda JC, Salaun B, Ji Y, Palmer DC, Monnot GC, Merck E, Boudousquie C, Utzschneider DT, Escobar TM, Perret R, Muljo SA, Hebeisen M, Rufer N, Zehn D, Donda A, Restifo NP, Held W, Gattinoni L, Romero P: MicroRNA-155 is required for effector CD8⁺ T cell responses to virus infection and cancer. *Immunity* 2013;38:742-753.
- 50 Tsai CY, Allie SR, Zhang W, Usherwood EJ: MicroRNA miR-155 affects antiviral effector and effector Memory CD8 T cell differentiation. *J Virol* 2013;87:2348-2351.
- 51 Cho S, Wu CJ, Yasuda T, Cruz LO, Khan AA, Lin LL, Nguyen DT, Miller M, Lee HM, Kuo ML, Broide DH, Rajewsky K, Rudensky AY, Lu LF: miR-23 approximately 27 approximately 24 clusters control effector T cell differentiation and function. *J Exp Med* 2016;213:235-249.
- 52 Liang Y, Pan HF, Ye DQ: microRNAs function in CD8⁺T cell biology. *J Leukoc Biol* 2015;97:487-497.
- 53 Smith NL, Wissink EM, Grimson A, Rudd BD: miR-150 Regulates Differentiation and Cytolytic Effector Function in CD8⁺ T cells. *Sci Rep* 2015;5:16399.
- 54 Zhong G, Cheng X, Long H, He L, Qi W, Xiang T, Zhao Z, Zhu B: Dynamically expressed microRNA-15b modulates the activities of CD8⁺ T lymphocytes in mice with Lewis lung carcinoma. *J Transl Med* 2013;11:71.
- 55 Ma F, Xu S, Liu X, Zhang Q, Xu X, Liu M, Hua M, Li N, Yao H, Cao X: The microRNA miR-29 controls innate and adaptive immune responses to intracellular bacterial infection by targeting interferon-gamma. *Nat Immunol* 2011;12:861-869.
- 56 Steiner DF, Thomas MF, Hu JK, Yang Z, Babiarz JE, Allen CD, Matloubian M, Brelloch R, Ansel KM: MicroRNA-29 regulates T-box transcription factors and interferon-gamma production in helper T cells. *Immunity* 2011;35:169-181.
- 57 Moffett HF, Cartwright ANR, Kim HJ, Godec J, Pyrdol J, Aijo T, Martinez GJ, Rao A, Lu J, Golub TR, Cantor H, Sharpe AH, Novina CD, Wucherpfennig KW: The microRNA miR-31 inhibits CD8⁽⁺⁾ T cell function in chronic viral infection. *Nat Immunol* 2017;18:791-799.
- 58 Podshivalova K, Salomon DR: MicroRNA regulation of T-lymphocyte immunity: modulation of molecular networks responsible for T-cell activation, differentiation, and development. *Crit Rev Immunol* 2013;33:435-476.
- 59 Banerjee A, Gordon SM, Intlekofer AM, Paley MA, Mooney EC, Lindsten T, Wherry EJ, Reiner SL: Cutting edge: The transcription factor eomesodermin enables CD8⁺ T cells to compete for the memory cell niche. *J Immunol* 2010;185:4988-4992.

- 60 Lazarevic V, Glimcher LH, Lord GM: T-bet: a bridge between innate and adaptive immunity. *Nat Rev Immunol* 2013;13:777-789.
- 61 Knudson KM, Pritzl CJ, Saxena V, Altman A, Daniels MA, Teixeira E: NFκB-Pim-1-Eomesodermin axis is critical for maintaining CD8 T-cell memory quality. *Proc Natl Acad Sci U S A* 2017;114:E1659-e1667.
- 62 Wissink EM, Smith NL, Spektor R, Rudd BD, Grimson A: MicroRNAs and Their Targets Are Differentially Regulated in Adult and Neonatal Mouse CD8⁺ T Cells. *Genetics* 2015;201:1017-1030.
- 63 Xin A, Masson F, Liao Y, Preston S, Guan T, Gloury R, Olshansky M, Lin JX, Li P, Speed TP, Smyth GK, Ernst M, Leonard WJ, Pellegrini M, Kaech SM, Nutt SL, Shi W, Belz GT, Kallies A: A molecular threshold for effector CD8(+) T cell differentiation controlled by transcription factors Blimp-1 and T-bet. *Nat Immunol* 2016;17:422-432.
- 64 Baitsch L, Baumgaertner P, Devereux E, Raghav SK, Legat A, Barba L, Wieckowski S, Bouzourene H, Deplancke B, Romero P, Rufer N, Speiser DE: Exhaustion of tumor-specific CD8(+) T cells in metastases from melanoma patients. *J Clin Invest* 2011;121:2350-2360.
- 65 Ohaegbulam KC, Assal A, Lazar-Molnar E, Yao Y, Zang X: Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway. *Trends Mol Med* 2015;21:24-33.
- 66 Wei J, Nduom EK, Kong LY, Hashimoto Y, Xu S, Gabrusiewicz K, Ling X, Huang N, Qiao W, Zhou S, Ivan C, Fuller GN, Gilbert MR, Overwijk W, Calin GA, Heimberger AB: MiR-138 exerts anti-glioma efficacy by targeting immune checkpoints. *Neuro Oncol* 2016;18:639-648.
- 67 Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, Gupta R, Tsai JM, Sinha R, Corey D, Ring AM, Connolly AJ, Weissman IL: PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. *Nature* 2017;545:495-499.
- 68 Jia L, Xi Q, Wang H, Zhang Z, Liu H, Cheng Y, Guo X, Zhang J, Zhang Q, Zhang L, Xue Z, Li Y, Da Y, Zhao P, Zhang R: miR-142-5p regulates tumor cell PD-L1 expression and enhances anti-tumor immunity. *Biochem Biophys Res Commun* 2017;488:425-431.
- 69 Wang X, Li J, Dong K, Lin F, Long M, Ouyang Y, Wei J, Chen X, Weng Y, He T, Zhang H: Tumor suppressor miR-34a targets PD-L1 and functions as a potential immunotherapeutic target in acute myeloid leukemia. *Cell Signal* 2015;27:443-452.
- 70 Pollizzi KN, Patel CH, Sun IH, Oh MH, Waickman AT, Wen J, Delgoffe GM, Powell JD: mTORC1 and mTORC2 selectively regulate CD8(+) T cell differentiation. *J Clin Invest* 2015;125:2090-2108.
- 71 Scholz G, Jandus C, Zhang L, Grandclement C, Lopez-Mejia IC, Soneson C, Delorenzi M, Fajas L, Held W, Dormond O, Romero P: Modulation of mTOR Signalling Triggers the Formation of Stem Cell-like Memory T Cells. *EBioMedicine* 2016;4:50-61.
- 72 Berezhnoy A, Castro I, Levay A, Malek TR, Gilboa E: Aptamer-targeted inhibition of mTOR in T cells enhances antitumor immunity. *J Clin Invest* 2014;124:188-197.
- 73 Backer RA, Helbig C, Gentek R, Kent A, Laidlaw BJ, Dominguez CX, de Souza YS, van Trierum SE, van Beek R, Rimmelzwaan GF, ten Brinke A, Willemsen AM, van Kampen AH, Kaech SM, Blander JM, van Gisbergen K, Amsen D: A central role for Notch in effector CD8(+) T cell differentiation. *Nat Immunol* 2014;15:1143-1151.
- 74 Boudousquie C, Danilo M, Pousse L, Jeevan-Raj B, Angelov GS, Chennupati V, Zehn D, Held W: Differences in the transduction of canonical Wnt signals demarcate effector and memory CD8 T cells with distinct recall proliferation capacity. *J Immunol* 2014;193:2784-2791.
- 75 Kondo T, Morita R, Okuzono Y, Nakatsukasa H, Sekiya T, Chikuma S, Shichita T, Kanamori M, Kubo M, Koga K, Miyazaki T, Kassai Y, Yoshimura A: Notch-mediated conversion of activated T cells into stem cell memory-like T cells for adoptive immunotherapy. *Nat Commun* 2017;8:15338.
- 76 Park EY, Chang E, Lee EJ, Lee HW, Kang HG, Chun KH, Woo YM, Kong HK, Ko JY, Suzuki H, Song E, Park JH: Targeting of miR34a-NOTCH1 axis reduced breast cancer stemness and chemoresistance. *Cancer Res* 2014;74:7573-7582.
- 77 Chen J, Zhang H, Chen Y, Qiao G, Jiang W, Ni P, Liu X, Ma L: miR-598 inhibits metastasis in colorectal cancer by suppressing JAG1/Notch2 pathway stimulating EMT. *Exp Cell Res* 2017;352:104-112.
- 78 Liang T, Guo Q, Li L, Cheng Y, Ren C, Zhang G: MicroRNA-433 inhibits migration and invasion of ovarian cancer cells via targeting Notch1. *Neoplasia* 2016;63:696-704.

- 79 Ghisi M, Corradin A, Basso K, Frasson C, Serafin V, Mukherjee S, Mussolin L, Ruggero K, Bonanno L, Guffanti A, De Bellis G, Gerosa G, Stellin G, D'Agostino DM, Basso G, Bronte V, Indraccolo S, Amadori A, Zanovello P: Modulation of microRNA expression in human T-cell development: targeting of NOTCH3 by miR-150. *Blood* 2011;117:7053-7062.
- 80 Ping Y, Liu C, Zhang Y: T-cell receptor-engineered T cells for cancer treatment: current status and future directions. *Protein Cell* 2017;10.1007/s13238-016-0367-1
- 81 Chong EA, Melenhorst JJ, Lacey SF, Ambrose DE, Gonzalez V, Levine BL, June CH, Schuster SJ: PD-1 blockade modulates chimeric antigen receptor (CAR)-modified T cells: refueling the CAR. *Blood* 2017;129:1039-1041.
- 82 Ali SA, Shi V, Maric I, Wang M, Stroncek DF, Rose JJ, Brudno JN, Stetler-Stevenson M, Feldman SA, Hansen BG, Fellowes VS, Hakim FT, Gress RE, Kochenderfer JN: T cells expressing an anti-B-cell maturation antigen chimeric antigen receptor cause remissions of multiple myeloma. *Blood* 2016;128:1688-1700.
- 83 Lim WA, June CH: The Principles of Engineering Immune Cells to Treat Cancer. *Cell* 2017;168:724-740.
- 84 Irving M, Vuillefroy de Silly R, Scholten K, Dilek N, Coukos G: Engineering Chimeric Antigen Receptor T-Cells for Racing in Solid Tumors: Don't Forget the Fuel. *Frontiers in Immunology* 2017;8:
- 85 Wu H, Neilson JR, Kumar P, Manocha M, Shankar P, Sharp PA, Manjunath N: miRNA profiling of naive, effector and memory CD8 T cells. *PLoS One* 2007;2:e1020.
- 86 Huang Q, Xia J, Wang L, Wang X, Ma X, Deng Q, Lu Y, Kumar M, Zhou Z, Li L, Zeng Z, Young KH, Yi Q, Zhang M, Li Y: miR-153 suppresses IDO1 expression and enhances CAR T cell immunotherapy. *J Hematol Oncol* 2018;11:58.
- 87 Ohno M, Ohkuri T, Kosaka A, Tanahashi K, June CH, Natsume A, Okada H: Expression of miR-17-92 enhances anti-tumor activity of T-cells transduced with the anti-EGFRvIII chimeric antigen receptor in mice bearing human GBM xenografts. *J Immunother Cancer* 2013;1:21.
- 88 Church SE, Jensen SM, Antony PA, Restifo NP, Fox BA: Tumor-specific CD4⁺ T cells maintain effector and memory tumor-specific CD8⁺ T cells. *Eur J Immunol* 2014;44:69-79.