miR-23a blockade enhances adoptive T cell transfer therapy by preserving immune-competence in the tumor microenvironment

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Adoptive T cell transfer therapy (ACT) is an attractive means of harnessing the immune system for cancer intervention. Due to their intrinsic ability to recognize and eradicate tumor cells, cytotoxic CD8+ T lymphocytes (CTLs) are primary candidates for use in ACT. In spite of their potential, the widespread clinical use of CTLs for ACT has been hampered by 2 main hurdles: (i) the difficulties in selecting, isolating and expanding tumor-reactive CTLs ex vivo to therapeutically-relevant numbers for reinfusion, and (ii) the vulnerability of these reinfused CTLs to the immunosuppressive nature of the tumor microenvironment.1 Recent advances in T-cell engineering — such as the forced expression of high-affinity tumor-specific T-cell receptors (TCRs), or chimeric antigen receptors (CARs), with optimal T-cell activation capabilities — have facilitated the generation of large quantities of autologous tumor-reactive T cells.2 Nevertheless, despite displaying potent antitumor activity ex vivo, exposure to the tumor microenvironment renders these engineered T cells dysfunctional in vivo.3 This calls for additional approaches capable of not only augmenting, but more importantly, preserving CTL antitumor function in immunosuppressive environments.

To address this quandary, we chose to adopt a microRNA-based approach for its two-fold advantages. First, compared to protein-based drug development processes, manipulating microRNAs through the use of microRNA mimetics and antisense oligonucleotides is much simpler, making microRNAs more convenient therapeutic targets.4,5 Second, microRNA targeting can be readily integrated with ACT, as microRNA levels can be altered during the window of ex vivo lymphocyte expansion.

We found that CTLs with contrasting cytotoxic potencies differed in their expression of a subset of microRNAs. This led us to hypothesize that these differentially-expressed microRNAs may regulate CTL effector function, and that the alteration of their expression levels may, in turn, impact CTL antitumor responses. Of these, miR-23a remained abundant in poorly-cytotoxic CTLs, but was dramatically downregulated in highly-cytotoxic CTLs. We next validated the causal role of miR-23a in CTL antitumor responses using the B16/F10 mouse model of melanoma coupled to pMel-1 CTLs that recognize the melanoma-associated antigen gp100. Overexpressing miR-23a in pMel-1 CTLs impaired their antitumor efficacy and resulted in increased tumor burden. We further demonstrated that miR-23a functionally inhibits CTLs by targeting PR domain containing 1, with ZNF domain (Pdml), better known as Blimp-1, a key transcription factor required for effector differentiation and optimal expression of cytotoxic mediators (e.g., granzyme B and IFNγ).6

Having identified miR-23a as an inhibitor of CTL cytotoxicity, we surmised that specific signals received by CTLs can control miR-23a expression to regulate their effector responses. Interestingly, we found that T cell-activating signals through the TCR and the immune-inhibitory cytokine transforming growth factor β (TGFβ) exert opposing effects on miR-23a levels. Counter-regulation between these 2 signals is achieved as both signaling pathways coalesce at the common signaling node cMyc, a transcriptional repressor of pri-miR-23a. Specifically, TCR stimulation induces cMyc to repress pri-miR-23a expression, while TGFβ dampens cMyc activity to release the transcriptional brakes on pri-miR-23a. As TGFβ represents one of the most significant immune barriers imposed by tumors,7 we were particularly intrigued by this novel post-transcriptional mechanism of TGFβ-mediated immunosuppression: the TGFβ—miR-23a—Blimp-1 axis. It is known that TGFβ triggers Smad2/3 activation to directly suppress the transcriptional activation of cytotoxic
Here, we revealed that TGFβ can concurrently up-regulate miR-23a in CTLs to inhibit Blimp-1 expression post-transcriptionally. Therefore, TGFβ executes immunosuppression through 2 arms: directly via Smad-dependent transcriptional repression of cytotoxic mediators, and indirectly via miR-23a-mediated post-transcriptional suppression of Blimp-1. Furthermore, our observation that TGFβ upregulates miR-23a in spite of optimal T cell-activating signals may serve to explain how tumor-specific T cells, including engineered CAR-modified T cells, are rendered dysfunctional by TGFβ.

To evaluate the relevance of miR-23a in the immune-pathogenesis of human cancers, we examined CD8$^+$ T cells isolated from the pleural effusion fluid [i.e., tumor-infiltrating lymphocytes (TILs)] and peripheral blood of patients with advanced lung cancer. Strikingly, miR-23a was upregulated in TILs, while Blimp-1 and its downstream target interferon γ (IFNγ) were downregulated. Moreover, miR-23a levels negatively correlated with Blimp-1 and IFNγ expression, corroborating our findings from mouse models that the tumor microenvironment causes CTL dysfunction through the induction of miR-23a. More importantly, the abundance of miR-23a in TILs highlights its potential as a clinically-relevant therapeutic target for the enhancement of ACT.

Based on the above findings, we theorized that inhibiting endogenous miR-23a could augment CTL effector responses, and more importantly, preserve their functionality upon TGFβ challenge. To test this, we blocked the function of endogenous miR-23a in CTLs either by pharmacological inhibition with an anti-miR-23a locked nucleic acid (LNA), or retroviral-transduction with a miR-23a decoy. In both cases, miR-23a-inhibited CTLs displayed augmented expression of key transcription factors and cytotoxic mediators in vitro, even in the presence of copious amounts of TGFβ. This motivated us to interrogate the utility of the miR-23a decoy retroviral vector as a gene therapy tool, as it could be feasibly incorporated into ACT for stable, long-lasting targeting of miR-23a. Indeed, miR-23a blockade in tumor-specific CTLs effectively retarded the progression of established tumors in mouse models. Inhibiting miR-23a in human CD8$^+$ T cells similarly augmented granzyme B expression, underscoring the translatability of this strategy.

Our findings collectively demonstrate a previously unappreciated post-transcriptional mechanism by which TGFβ mediates tumor immune-evasion and provide a miRNA-based strategy to subvert immunosuppressive barriers during ACT (Fig. 1). Furthermore, we have demonstrated the clinical-relevance and translational potential of miR-23a blockade in human CD8$^+$ T cells. Our study thereby offers miR-23a inhibition as a translational strategy to preserve CTL immune competence within the tumor microenvironment and subvert the immunosuppressive effect of TGFβ.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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