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RESEARCH HIGHLIGHT

Crucial role of miR-31 in induction of CD8$^+$ T-cell exhaustion and reinforcement of type 1 interferon signaling

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CD8$^+$ T cells play a key role in the eradication of viral (or bacterial) infections and cancers. When encountering viral antigens derived from an ongoing infection, they quickly undergo a series of activation events required for clonal expansion and acquisition of effector functions for clearance of viral infection. As the infection resolves, the size of the virus-specific T-cell population also shrinks, with effector T cells undergoing activation-induced cell death. Still, a small number of activated virus-specific T cells survive even after clearance of the infection as 'memory' T cells, so that the host immune system can handle subsequent infections with the same virus more robustly.[1] While the CD8$^+$ T-cell-mediated antiviral immune mechanism is generally fairly effective in eradicating viral infection, certain viruses, for example, lymphocytic choriomeningitis virus (LCMV) clone 13, have a mechanism to evade the immune reaction, resulting in persistent (chronic) infection. In contrast to conventional CD8$^+$ memory T cells, virus-specific CD8$^+$ T cells in the host with a persistent (chronic) infection are often found to be functionally impaired; this type of immune dysfunction is known as 'T-cell exhaustion.'[2] The high-level expression of diverse inhibitory receptors (for example, PD-1, CTLA-4, LAG-3 and TIM-3 and so on) is a hallmark of exhausted T cells; the high-level expression of diverse inhibitory receptors is caused by alterations in the epigenetical and transcriptional mechanisms. In addition, it is generally accepted that long-term exposure of activated T cells to type I interferon (IFN-α/β) is closely linked to T-cell exhaustion.[2]

MicroRNAs (miRNAs) are 21–23 base pair-long non-coding RNAs. The miRNA genes are located in intronic, exonic or 5′ (or 3′) untranslated regions of genes. They are first synthesized by RNA polymerase II as 500–3000 nucleotide-long pri-miRNAs that are processed by the Drosha–DGBC8 complex in the nucleus into 60–100 nucleotide-long double-stranded pre-miRNAs with hairpin structure and then further in the cytoplasm by the Dicer complex. The resulting mature miRNA forms a complex with a protein called Argonaute and is assembled into the RNA-induced silencing complex (RISC) with a target mRNA; miRNA facilitates the degradation of target mRNAs or interferes with ribosome binding via formation of RISC. The importance of miRNAs in regulating various cellular mechanisms, including cell growth and differentiation, has been underlined in numerous studies. The importance of miRNAs in regulation of T-cell immunity has been also documented in several recent studies.[3] For example, a study using T-cell-specific miR-29 knockout mice has demonstrated the importance of miR-29 in regulating the expression of key transcription factors (that is, T-bet and EOMES) that are imperative for the development of CD8$^+$ T-cell effector functions and the formation of CD8$^+$ T-cell memory.[4]

A new study by Moffett *et al.*[5] published in a recent issue of *Nature Immunology* also highlights the role of a specific miRNA, designated as miR-31, in shaping the fate of activated CD8$^+$ T cells. In an effort to identify miRNAs expressed in CD8$^+$ T cells only after activation, the authors carried out miRNA profiling with resting and activated (anti-CD3 plus anti-CD28 mAbs-treated) CD8$^+$ T cells, and discovered that expression of miR-31 was rapidly induced after activation; while both activated CD4$^+$ T cells and NK cells also expressed miR-31, their expression levels were over tenfold lower than those of CD8$^+$ T cells. They also found that miR-31 expression was dependent on the signaling mechanism involving Ca^{2+} and NFAT1. Gene-chip experiments showed that ectopic expression of miR-31 in primary T cells using a lentivirus expression system resulted in a

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decrease in the levels of multiple mRNAs (for example, *Ppp6C*, *Lats2* and *Stk 40*). It is also of note that ingenuity pathway analysis of all mRNAs in $CD8^+$ T cells transfected with lenti-miR-31 and with the control vector, respectively, revealed that the ectopic expression of miR-31 caused alteration (upregulation) in the levels of mRNAs specifically involved in type 1 IFN function involving *Ifna2* mRNA itself and *Irf3* and *Irf7* mRNAs.

Next, the authors examined the effect of an miR-31 deficiency on the repertoire of mRNAs in activated $CD8^+$ T cells using the mice with a targeted germ-line deletion of the miR-31 gene. In summary, they found that miR-31 deficiency resulted in the increase in the expression of a set of genes involved in effector T-cell function, such as perforin, several granzymes and osteopontin, and also the reduction in the expression of a set of genes involved in T-cell exhaustion, including intracellular metallothioneins, transcription factor c-Maf and the receptor for prostaglandin E2. Notably, such effects became even more prominent when miR-$31^{-/-}$ $CD8^+$ T cells were activated in the presence of IFN-β. Moreover, the data obtained from gene-set-enrichment analysis showed that the pattern of differential gene expression 'before vs after' treatment of activated $CD8^+$ T cells with IFN-β has a strong similarity to those of 'LCMV Armstrong vs clone 13' and 'effector vs exhausted T cells.' In addition, the results from experiments using 7678 mouse T-cell hybridoma transfected with miR-31 target gene-specific shRNA, that is, shRNAs for *Ppp6c*, *Lats2*, *Stk40* and *Sh2d1a*, suggested that the transcript of *Ppp6c*, the gene encoding a phosphatase known to downregulate type 1 IFN (IFN-α/β) signaling through Map3k7, was a potential target for miR-31 responsible for increased sensitivity of miR-31-deficient T cells to IFN-β.

IFN-α/β play positive roles in early stage of antiviral immunity by facilitating $CD8^+$ T-cell priming. Yet, as noted, they also play an active role in the induction of T-cell exhaustion during chronic viral infection.[6] Thus, the authors examined whether miR-31 deficiency had an effect on the eradication of the LCMV clone 13 infection whose infection in wild-type mice normally leads to persistent infection and T-cell exhaustion. Of interest, it was found that miR-$31^{-/-}$ mice resolved the LCMV clone 13 infection much faster and more effectively than wild-type mice even though the initial disease course appeared similar in both mice. Likewise, the number of LCMV-specific $CD8^+$ T cells in the blood was found much larger in miR-$31^{-/-}$ mice than in wild-type mice when checked on 20 day after infection. In addition, LCMV-specific $CD8^+$ T cells in miR-$31^{-/-}$ mice were also found to express an effector T cells marker (that is, KLRG1) at a higher level than the T cells in wild-type mice; conversely, they expressed an inhibitory receptor (that is, PD-1) at a lower level than the T cells in wild-type mice. Notably, the difference in effectiveness for resolving viral infection made by miR-31 deficiency appeared to apply only to chronic virus infection such as LCMV clone 13 infection as no clear difference was observed in eradicating influenza virus infection between the miR-$31^{-/-}$ and wild-type mice.

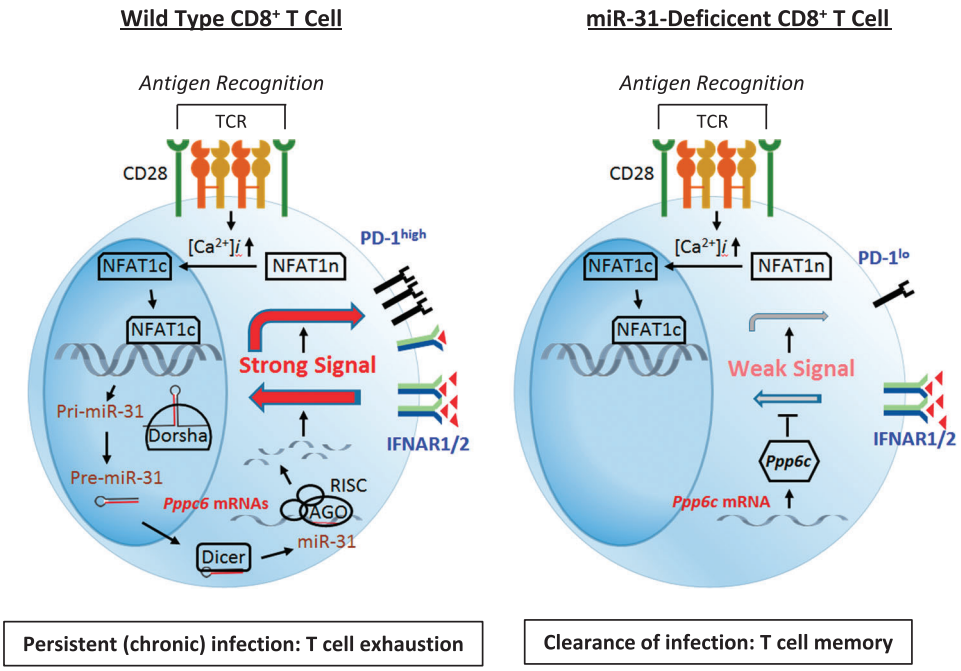

Figure 1 In wild-type mice, miR-31 expressed in $CD8^+$ T cells following TCR recognition and costimulatory receptor–ligand interactions binds to its target mRNAs, including *Ppp6c* mRNA, which encodes a phosphatase involved in downregulation of type I IFN (IFN-α/β) signaling. As a result, the magnitude of type I IFN signal is escalated, resulting in increase in the expression of T-cell inhibitory receptors such as PD-1 that plays a critical role in the induction of T-cell exhaustion. In miR-31-deficient mice, the level of Ppp6c mRNA remains high, and the magnitude of type I IFN signal is kept under control. As a result, $CD8^+$ T cells remain functionally competent.

In order to verify that the changes in the pattern of LCMV clone 13 infection observed in miR-31$^{-/-}$ mice were driven by T-cell-intrinsic effects, the authors used Mir-31$^{fl/fl}$Cd4^{cre} mice where targeted deletion of the miR-31 gene occurred only in T cells. Expectedly, the LCMV clone 13 infection was rapidly resolved in the Mir-31$^{fl/fl}$Cd4^{cre} even though the same infection caused the chronic disease in wild-type mice. The frequency of LCMV-specific CD8$^+$ T cells in the blood was also larger in miR-31$^{fl/fl}$Cd4^{cre} mice than in wild-type mice when checked on 20 days after infection. In addition, LCMV-specific T cells in Mir-31$^{fl/fl}$Cd4^{cre} mice still held competent T-cell functions 20 days after virus infection while LCMV-specific T cells in wild-type mice already turned functionally exhausted.

The study by Moffett *et al.* summarized above (Figure 1) convincingly states that a specific miRNA designated as miR-31 is directly involved in the down-regulation of CD8$^+$ T-cell effector function and the induction of T-cell exhaustion by type 1 IFNs (IFN-α/β). This study also provides a new insight for the development of a novel antiviral therapy. While the conclusion of the study was mainly drawn from the observations made from LCMV clone 13 infection, it also has a huge implication in CD8$^+$ T-cell-mediated antitumor immunity. Tumor-specific CD8$^+$ T cells in cancer patients are often found to express a high level of PD-1 and to be functionally impaired (exhausted). While anti-PD-1 (or anti-PD-L1) mAb therapy is largely successful and is becoming well established as a standard therapy for various cancers,[2] it is also true that many cancers are refractory to the mAb therapy. Thus, development of a method to alter the level of miR-31 in CD8$^+$ T cells may provide another strategy to rejuvenate the antitumor T-cell immunity in the cancer patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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