MicroRNA-150 regulates the cytotoxicity of natural killers by targeting perforin-1☆

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Background: Perforin-1 (Prf1) is the predominant cytolytic protein secreted by natural killer (NK) cells. For a rapid immune response, resting NK cells contain high Prf1 mRNA concentrations while exhibiting minimal cytotoxicity caused by a blockage of Prf1 protein synthesis, implying that an unknown posttranscriptional regulatory mechanism exists.
Objective: We sought to determine whether microRNA-150 (miR-150) posttranscriptionally regulates Prf1 translation in both mouse and human NK cells at rest and at various time points after activation.
Methods: Mouse NK cells with a targeted deletion of miR-150 (miR-150$^{-/-}$ NK cells), primary human NK cells, and NK92 MI cells were used to investigate the role of miR-150 in NK cells. NK cell cytotoxicity assays and Western blotting proved that activated miR-150$^{-/-}$ NK cells expressed upregulated Prf1, augmenting NK cell cytotoxicity. When immunodeficient mice were injected with miR-150$^{-/-}$ NK cells, there was a significant reduction in tumor growth and metastasis of B16F10 melanoma.
Results: We report that miR-150 binds to 3′ untranslated regions of mouse and human Prf1, posttranscriptionally downregulating its expression. Mouse wild-type NK cells displayed downregulated miR-150 expression in response to IL-15, which led to corresponding repression and induction of Prf1 during rest and after IL-15 activation, respectively.
Conclusion: Our results indicate that miR-150 is a common posttranscriptional regulator for Prf1 in mouse and human NK cells that represses NK cell lytic activity. Thus the therapeutic control of miR-150 in NK cells could enhance NK cell–based immunotherapy against cancer, providing a better clinical outcome. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *miR-150, NK cells, perforin-1, NK cell cytotoxicity, post-transcriptional regulation, immunotherapy, tumor growth and metastasis*

Natural killer (NK) cells kill target cells predominantly by secreting granule toxins, including perforin-1 (Prf1) and granzymes.[1-3] Prf1 is a pore-forming protein, and granzymes are structurally related serine proteases that lyse target cell protein at specific aspartate residues.[4,5] Prf1 disturbs the target cell membrane and facilitates the entry, trafficking, or both of granzymes.[6] Unlike multiple granzymes, which can compensate each other, Prf1 is encoded by a single gene and does not have any functional redundancy.[7]

NK cells kill target cells "naturally" without prior antigen-specific recognition, allowing for rapid induction of lytic activity.[8] For prompt immune responses, resting NK cells are in a "prearmed" state, containing high concentrations of Prf1 mRNA, but they are minimally cytotoxic because of a blockage of Prf1 translation.[9] On target recognition, activated NK cells immediately arm themselves with preformed Prf1 mRNA, correlating with an increase in Prf1 protein levels. Therefore Prf1 is posttranscriptionally regulated by unknown regulators in resting NK cells.

MicroRNAs (miRNAs) are small noncoding RNAs of approximately 22 nucleotides that function as posttranscriptional inhibitors complementary to the 3′ untranslated region (UTR) of their target mRNAs.[10] Over the past few years, emerging data have implied that endogenously generated miRNAs are posttranscriptional regulators of immune cell development and function.[11] miR-150 has been identified as a lymphocyte-specific miRNA because it is predominantly expressed in the lymph nodes, spleen, and thymus and is highly upregulated during lymphocyte maturation. miR-150 expression increases sharply in mature B and T lymphocytes, as well as in mature NK and invariant NK T cells, although not in their progenitors.[12-16]

Although miR-150 expression is upregulated during lymphocyte maturation, it is downregulated again during the activation of mature B and T cells. Xiao et al[14] reported that miR-150 expression was downregulated in activated B cells and that miR-150–deficient mice exhibit enhanced humoral and T cell–dependent antibody responses with increased steady-state immunoglobulin levels. Collectively, miR-150 appears to be inversely associated

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Abbreviations used
AANAT: Aralkylamine N-acetyltransferase
DMEM: Dulbecco modified Eagle medium
E/T: Effector/target
GAPDH: Glyceraldehyde-3-phosphate dehydrogenase
GzmB: Granzyme B
HEK: Human embryonic kidney
miRNA: MicroRNA
NeoR: Neomycin resistance gene
NK: Natural killer
PI: Propidium iodide
Prf1: Perforin-1
UTR: Untranslated region
WT: Wild-type

with the immunologic functions of activated B and T cells, but a relationship between miR-150 and the activation of NK cells has not yet been shown.

In this study we report that miR-150 binds to the 3′ UTR of mouse and human Prf1, posttranscriptionally downregulating its expression. Mouse wild-type (WT) NK cells exhibited biphasic upregulated and downregulated miR-150 expression in response to IL-15, which led to corresponding repression and induction of Prf1 during rest and after IL-15 activation, respectively. Primary human NK cells also display downregulated miR-150 and augmented Prf1 in response to IL-15. Our results suggest that miR-150$^{-/-}$ NK cells might provide clinical benefit to minimize spontaneous activation in resting NK cells while maximizing cytotoxicity in activated NK cells. Therapeutic modulation of miR-150 might be a promising new approach for enhancing NK cell–mediated immunotherapy to treat various human pathologies.

METHODS

For a detailed description of the methods used in this study, see Tables E1 and E2 and the Methods section in this article's Online Repository at www.jacionline.org.

RESULTS

miR-150 expression is inversely proportional to Prf1 protein expression in both mouse and human NK cells during IL-15 activation

The expression of miR-150 in mouse WT NK cells exhibited a biphasic pattern during IL-15 stimulation. The miR-150 level was sharply increased nearly 2-fold at 4 hours and maintained for a time. Then its level was markedly decreased to less than the level of resting NK cells at 24 hours and further downregulated at 72 hours. The miR-150 level was negatively associated with expression of Prf1 protein (Fig 1, *A*). Primary human NK cells showed gradually decreased expression of miR-150 and increased levels of Prf1 protein in response to IL-15 (Fig 1, *B*). In both mouse and human NK cells, relatively high abundance of miR-150 and sustained expression of Prf1 and granzyme B (GzmB) protein were observed during the first 6 hours of IL-15 stimulation. Then Prf1 and GzmB protein expression started to increase, accompanied by reduced expression of miR-150 at 24 hours (Fig 1, *C* and *D*, top). In addition, GzmB mRNA expression was notably increased in a time-dependent manner correlating with an increase in GzmB protein levels, but abundant mRNA of Prf1 in resting NK cells remained largely unchanged during IL-15 activation in both mouse and human NK cells (Fig 1, *C* and *D*, bottom). This implies that Prf1 could be posttranscriptionally repressed by miR-150 in NK cells at rest and earlier time points after IL-15 activation.

miR-150$^{-/-}$ NK cells have augmented Prf1 protein expression and enhanced NK cell cytotoxicity

Resting WT and miR-150$^{-/-}$ NK cells minimally expressed Prf1 protein, but Prf1 protein was amplified in miR-150$^{-/-}$ NK cells at 48 and 72 hours of IL-15 stimulation. However, Prf1 mRNA abundance did not change over time (Fig 2, *A*). After 48 hours of IL-15 stimulation, miR-150$^{-/-}$ NK cells exhibited enhanced cytotoxicity by approximately 2-fold at an effector/target (E/T) ratio of 5:1 (Fig 2, *B*). Collectively, abundant Prf1 mRNA was posttranscriptionally suppressed in resting NK cells, but miR-150$^{-/-}$ NK cells augmented Prf1 protein expression and cytotoxicity on IL-15 activation.

WT and miR-150$^{-/-}$ NK cells have similar NK cell receptor profiles, degranulation, and death receptor/ligand interactions

NK cells express various receptors to recognize target cells. Therefore we examined a broad range of activating NK cell receptors (NKG2D, NKp46, and Ly49D), inhibitory receptors (Ly49C/I, Ly49G2, and Ly49A), IL-15 receptors (CD122 and CD132), and chemokine receptors (CXCR5 and CCR6). IL-15–activated WT and miR-150$^{-/-}$ NK cells displayed similar frequencies in the receptor repertoire, including IL-15 receptors, and little or no chemokine receptor expression (Fig 3, *A*). It led to similar NK cell degranulation evidenced by the intensity of CD107a in WT and miR-150$^{-/-}$ NK cells after treatment with antibody against NKp46 in the presence or absence of target cells (Fig 3, *B*). NK cell cytotoxicity can also be mediated in the absence of Prf1 through engagement of death receptors (eg, Fas/CD95) on target cells through their cognate ligands (eg, Fas ligand) on NK cells.[17] We investigated the levels of 2 key effector ligands, Fas ligand and TNF-related apoptosis-inducing ligand, and the death receptor CD95 in IL-15–activated NK cells. miR-150 had no profound effects on the expression of these ligands and receptors (Fig 3, *A* and *C*). These data suggest that the augmented cytotoxicity of miR-150$^{-/-}$ NK cells is caused predominantly by enhanced Prf1 and not by significant changes in NK cell receptor profiles, IL-15 receptor–mediated signaling pathways, or death receptor/ligand interactions.

miR-150$^{-/-}$ NK cells show that potent lytic granules hit at the immunologic synapse

NK cells are functionally heterogeneous, and thus only a small portion of NK cells kill target cells.[18] Lytic granules of NK cells were labeled with LysoSensor Green, and target cells were labeled with DDAO-SE and then cocultured in propidium iodide (PI)–containing media to assess the dynamics of individual NK cell cytotoxicity. Larger amounts of lytic granules containing Prf1 and GzmB were found in miR-150$^{-/-}$ NK cells