Functional Role of MicroRNAs in Thymocyte Development

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Keywords

miRNAs · Thymocytes · Development

Abstract

MicroRNAs (miRNAs) are a class of endogenous noncoding single-stranded RNAs widely distributed in eukaryotes, which can modulate target gene expression at posttranscriptional level and participate in cell proliferation, differentiation, and apoptosis. Related studies have shown that miRNAs are instrumental to many aspects of immunity, including various levels of T-cell immunity. In addition, multiple miRNAs have been ascribed key roles in T-cell development, differentiation, and function. In this review, we highlight the current literature regarding the functional role of miRNAs at various stages of thymocyte development. A better understanding of the relationship between miRNAs and thymocyte development is helpful for the exploration of the exact roles of miRNAs in the development and function of the immune system, as well as related clinical diseases.

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Introduction

The thymus, a central immune organ in humans and other mammals, is an important place for the development, differentiation, and maturation of immune cells. T lymphocytes, referred to as T cells, are derived from the early T-lineage precursor of the bone marrow or embryonic liver lymphoid stem cells, which develop and mature in the thymus and then migrate into peripheral immune organs or tissues to perform their biological functions [1–3]. During the development of the thymus, T cells can be divided into 3 stages based on the differential expression of CD4 and CD8 coreceptors (Fig. 1). The early T-cell phenotype consists of CD4[−]CD8[−] double-negative cells (DN), which mainly differentiate in the cortical region; subsequently, DN T cells differentiate into CD4⁺CD8⁺ double-positive cells (DP), begin to express T-cell receptor (TCR), and gradually migrate into the medulla. Furthermore, the MHC-restricted recognition ability is obtained by pos-

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Fig. 1. The basic process of thymocyte development. Schematic representation showing the different cell surface markers expressed in key stages of T-cell development in mouse thymus, including double negative (DN), double positive (DP), and CD4 and CD8 single positive (SP) stages. BM, bone marrow; ETP, early T-lineage precursor.

Fig. 2. The role of different miRNAs in thymocyte development. Schematic is shown for the role of several miRNAs in thymocyte development. These miRNAs are involved directly or indirectly (purple targets) in the regulation of crucial aspects of T-cell activation, proliferation, and differentiation as depicted.

itive selection, and the tolerance to its own antigen is obtained by negative selection. Finally, these cells develop into mature T cells with only CD4 or CD8 single-positive (SP) expression, then migrate out of the thymus, and move to the peripheral lymphoid organs or tissues to settle and perform a corresponding immune function.

Studies have shown that microRNAs (miRNAs) are vital to many aspects of immunity, including various levels of T-cell immunity [4–9]. miRNAs are small, non-coding, single-strand ~22 nucleotide (nt)-long RNAs generated from primary miRNAs (pri-miRNAs) containing a stem loop structure, which are processed in the nucleus by the enzymes Drosha and DiGeorge syndrome critical region 8 (DGCR8) into shorter pre-miRNAs [10]. The pre-miRNA is subsequently processed in the cytoplasm by Dicer, resulting in RNA duplex of

20–22 nt [11, 12]. The RNA duplex is incorporated into the RNA-induced silencing complex, where one of the strands undergoes degradation while the other forms the mature miRNA [13]. Mature miRNAs can bind to the 3'-untranslated region of complementary or partially complementary target gene mRNA and promote the degradation of target gene mRNA at posttranscriptional level or mediate its translation inhibition, thus exerting a wide range of physiological regulatory functions [14]. In humans, more than 1,000 miRNA family members have been reported to participate in important biological processes such as cell development, differentiation, proliferation, apoptosis, tumorigenesis, and development. Accumulating evidence has shown that miRNAs play an important regulatory role in the development of thymocytes, indicating their importance in the development of the immune system.

miRNAs and T-Cell Development

Dynamic regulation of miRNA expression in sequential stages of thymocyte development is well documented [15]. An overall increase in miRNA levels at early stages of T-cell maturation (DP stage) with an increase in total cellular RNA content [15] suggests that its expression is associated with thymocyte development. Cobb et al. [16] further found that in the early stage of thymocyte development (DN to DP stage), conditional deletion of *Dicer*, a key enzyme required for miRNA maturation, would reduce the cellularity of DP thymocytes, which was driven mainly by reduced numbers and survival of $\alpha\beta$ T cells, whereas the number of $\gamma\delta$ T cells was not affected. Notably, the number of CD4 and CD8 SP T cells in the spleen and the total number of CD3⁺ T cells in the peripheral blood decreased obviously after conditional deletion of *Dicer* [16, 17]. In addition, individual miRNAs showed dynamic changes in different thymocyte subsets (DN, DP, and SP), and the increase in single miRNAs during thymocyte development was negatively correlated with the deletion of target genes [15, 18]. These studies indicated that specific miRNAs were involved in the development of thymocytes (Fig. 2).

miR-181

In an miR-181a-overexpressing mouse model, the number of T cells in the peripheral circulation decreased significantly, especially the CD8⁺ T-cell number was reduced by 90% [19], suggesting that miR-181a may play an important role in the development of T cells. Functional analysis of miR-181a-1 and miR-181c by Liu et al. [20] showed that miR-181a-1 was ectopically expressed in thymic progenitor T cells and promoted the differentiation of CD4⁺CD8⁺DN to CD4⁺CD8⁺DP cells, while miR-181c did not have such a function, it might be that the unique pre-miRNA stem loop nt sequence of miR-181a-1 determined its functional specificity. Furthermore, Neilson et al. [15] found that miR-181a specifically increased in the CD4⁺CD8⁺DP stage of thymocyte development, and that miR-181a could inhibit the expression of B-cell lymphoma/leukemia 2 (*Bcl-2*) and CD69, for example, thereby affecting their coordination and participation in the positive selection process of CD4⁺CD8⁺DP T cells [21].

The sensitivity of TCR plays an important role in the positive and negative selection of T cells. Li et al. [22] confirmed that after the upregulation of miR-181a expression in mature T cells, the sensitivity of T cells to antigenic peptides would increase, and, meanwhile, the intracellular calcium ion outflow and cytokine IL-2 production

would be enhanced; downregulation of miR-181a expression in immature T cells reduced T-cell sensitivity to antigenic peptides and impaired negative and positive selection of thymocyte development; in addition, the quantitative regulation of the sensitivity of miR-181a to T cells enabled mature T cells to recognize an antagonist (inhibitory antigen peptide) as an agonist. Li et al. [22] further found that miR-181a could increase the homeostasis of the phosphorylated intermediate by inhibiting the expression of tyrosine and serine phosphatase, thereby reducing the signal threshold of TCR and enhancing its sensitivity. Subsequent analysis showed that miR-181a downregulated the expression of multiple phosphatase genes (such as SHP-2, PTPN22, DUSP5, and DUSP6). Importantly, the high expression of miR-181a was associated with the sensitivity of immature T cells, which suggested that miR-181a might act as an intrinsic “rheostat” for antigenic sensitivity during the development of T cells. Ebert et al. [23] also found that inhibition of miR-181a expression promoted T-cell responses to participate in the positive selection of its own peptides, leading to the maturation of T cells, while miR-181a could prevent the deletion of moderate affinity clone by regulating the threshold of the thymocyte TCR signal, ensuring the smooth progress of positive selection.

miR-150

The miRNA expression profile of humans and mice showed that the level of miR-150 was upregulated during T-cell maturation [18, 24]. Zhou et al. [25] further discovered low expression of miR-150 in the CD4⁺CD8⁺DN stage of thymocytes, moderate expression in the CD4⁺CD8⁺DP stage and CD8⁺ T cells, while expression was high in CD4⁺ T cells – this dynamic change in miR-150 suggested that it might have a regulatory effect on thymocyte development. In miR-150 transgenic mice, overexpression of miR-150 blocked the development of murine thymocytes, especially the differentiation of DN3 to DN4, which ultimately led to a decrease in the number of CD4⁺ and CD8⁺ T cells [24]. In terms of the mechanism, the transcription factor c-myeloblastosis (*c-Myb*) was an important target molecule of miR-150, and its expression is downregulated when miR-150 was overexpressed in immature T cells [24]. In addition to *c-Myb*, NOTCH3 was also an important new target molecule of miR-150, which was the main regulatory factor of T-cell differentiation and can reduce the proliferation and survival of T cells [18]. These research works suggested that miR-150 was instrumental to T-cell maturation.

Other studies have shown that miR-150 is also involved in the development of different functional T-cell subsets. For example, the invariant nature killer T (iNKT) cells are a unique subset of T lymphocytes, which express a constant TCRV α 14 and a partial marker of NK cells, CD161 (NK1.1), and NK cell receptors, NKR-P1C. Zheng et al. [26] demonstrated that miR-150 expression was up-regulated during iNKT cell maturation and activation, and iNKT cell maturation in the thymus was impaired in the miR-150-knockout mouse model. Subsequently, they also confirmed that miR-150 knockout could change iNKT cell maturation and function by using adoptive cell transfer of bone marrow cells. Furthermore, Bezman et al. [27] studied miR-150 overexpression in mice and came to similar conclusions. The above studies indicate that miR-150 played important roles in the development and function of iNKT cells. However, whether it is also involved in the development of other T-cell subsets remains to be elucidated.

miR-146

Under normal physiological conditions, miR-146a was expressed in various immune cells, including T cells [28]. It has been reported that overexpression of miR-146a in bone marrow lymphoid progenitor cells might impair the development of hematopoietic stem/progenitor cells, eventually resulting in a decreased number of CD4⁺ T cells in the peripheral blood [29]. In addition, Kirigin et al. [30] found that the expression level of miR-146a was different in thymocytes at different developmental stages of the thymus. Li et al. [31], using miR-146a overexpression in a mouse model, found that overexpression of miR-146a could promote T-cell proliferation and reduce T-cell numbers. More importantly, in this murine model, positive selection of thymocytes, a key process for central tolerance, was weakened, resulting in an increase in CD4⁺CD8⁺DP T cells and a decrease in CD4⁺SP and CD8⁺SP T cells. Moreover, the maturation process of CD8⁺SP T cells was weakened, which led to a more severe loss of CD8⁺SP T cells than CD4⁺SP T cells. The above studies showed that miR-146a can regulate thymocyte development through the development of bone marrow lymphocyte progenitor cells and thymic cells. However, the related target molecular mechanism remains to be further studied.

miR-17-92

MiR-17-92 is a cluster of 6 miRNAs (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92-1). In the bone marrow, overexpression of miR-17-92 in lym-

phoid cells could lead to excessive cell proliferation, which may further affect the migration of lymphoid cells into the thymus, leading to dysplasia of thymocytes. During thymus development, Xiao et al. [32] used the mouse model that overexpressed miR-17-92 in the DN1 stage of thymocyte development and found that the number of peripheral T lymphocytes in this mouse model was significantly changed, especially CD4⁺ T cells, suggesting that miR-17-92 was involved in the thymocyte development process. Regelin et al. [33] further used miR-17-92 Δ/Δ mice and found that thymocytes lacking miR-17-92 had serious defects in early development, especially the differentiation process of DN to DP. These studies suggested that miR-17-92 played a crucial role in the regulation of thymocyte development and might be more related to the development of CD4⁺ T cells. However, its exact role and related mechanisms in various stages of thymocyte development remain unclear.

miR-155

Natural regulatory T cells (nTreg) are CD4⁺ T cells produced by lymphocytes during thymus maturation, which express CD25 molecules. These cells are transported to peripheral lymphoid organs or tissues through the blood flow after thymus maturation and perform a corresponding immune function. Studies have found that miR-155 plays an important regulatory role in the development of nTreg cells [34–36]. In miR-155-knockout mice, the number of Treg cells in the thymus and peripheral lymphoid organs was decreased. Moreover, the proliferation capacity of Treg cells lacking miR-155 was also significantly weakened [34, 37]. IL-2 is a key factor in the development and survival of Treg cells [38–40]. Further studies have found that miR-155 can promote Treg cells in the thymus by enhancing the sensitivity of Treg to IL-2 and increasing signal transduction and activator of transcription 5 (STAT5) signaling pathways, thereby increasing the proliferation and survival of Treg cells in the thymus and periphery [37]. Interestingly, Kohlhaas et al. [34] also found that forkhead box protein 3 (Foxp3) can regulate the expression of miR-155 in nTreg cells. Given the importance of the STAT5 signaling pathway in Foxp3 expression [41], we hypothesized that there is a regulatory loop between miR-155, STAT5, and Foxp3 to regulate the development of nTreg cells. In addition, Sánchez-Díaz et al. [42] also found that the related pathway of C-type lectin could also regulate thymus development of Treg cells through the expression of miR-155, suggesting the complexity of the miR-155 molecule involved in thymic Treg development.

Table 1. miRNAs, their targets, and their effects in the development of thymocytes

miRNA	Target	Effects	Species	Ref.
miR-10a	Bcl-6	Restricts Treg cell differentiation into Th17 cells	Mouse	50
miR-17–92	IL-7R	Reduces cell survival of common lymphoid progenitors and thymocytes at the DN to DP transition	Mouse	27
	CREB1 TGFβRII PTEN	Inhibits formation of iTreg	Mouse	51
miR-29a	Dkk Kremen2 sFRP2	Promotes canonical Wnt signaling in osteoblasts	Human	52
miR-34	Wnt1 Wnt3 β-Catenin	Suppresses canonical Wnt signaling in epithelial and breast cancer cells	Human	53
miR-125b	Bmf KLF13	Regulates HSC survival and promotes lymphoid fate decisions at the level of the HSC	Mouse	39
miR-126	IRS-1	Regulates the development of CD4 ⁺ SP cells	Mouse	43
miR-133b	Th-POK	Regulates the differentiation of NKT17 cells in the thymus	Mouse	42
miR-142	Cdkn1b	Controls the early and mature thymocyte proliferation	Mouse	38
miR-146a	Gimap4 STAT1	Impairs the positive selection during T-cell development	Mouse	25
		Inhibits IFNγ signaling in Treg	Mouse	54
miR-150	NOTCH3 c-Myb	Regulates pre-TCR and NFκB signaling	Human	12
		Blocks DN3 to DN4	Mouse	18
miR-155	SOCS1	Promotes Treg survival and thymic development	Mouse	28, 31
	CD69	Promotes Treg cell development and homeostasis	Mouse	36
	Itk	Mediates TGFβ signaling in intestinal lamina propria T cells	Human	55
	No report	Promotes Th17 polarization	Mouse	56
	Ets1, Itk	Blocks the maturation of iNKT cells	Mouse	37
miR-181a	PTPN22 DUSP5/6 SHP-2	Increases TCR sensitivity and regulates positive selection	Mouse	16, 17
miR-205	Forkhead box N1	Promotes T cell development	Mouse	40
miR-223	E2F1	Contributes to the development of T cells	Mouse	41
	MEF2C TOX			
miR-873	Foxo1	Facilitates the differentiation of CD4 ⁺ T cells into Th17 lineage	Human	57
miR-4281	Foxp3	Accelerates the differentiation of human naive cells to iTreg	Mouse	36

Bcl-6, B cell lymphoma-6; IL-7R, interleukin-7 receptor; CREB1, cAMP-responsive element binding protein 1; TGFβRII, transforming growth factor β receptor II; PTEN, phosphatase and tensin; Dkk, dickkopf WNT signaling pathway inhibitor; Kremen2, krigle-containing transmembrane protein 2; sFRP2, secreted frizzled-related protein 2; Wnt1, wingless type 1; Wnt3, wingless type 3; Bmf, Bcl2 modifying factor; KLF13, Kruppel-like factor 13; IRS-1, insulin receptor substrate-1; Th-POK, T-helper-inducing POZ/Kruppel-like factor; Cdkn1b, cyclin-dependent kinase inhibitor 1B; Gimap4, GTPase IMAP family member 4; STAT1, signal transduction and activator of transcription 1; c-Myb, c-myeloblastosis; SOCS1, suppressor of cytokine signaling 1; Itk, interleukin-2-inducible T-cell special kinase; Ets1, E26 transformation-specific factor-1; PTPN22, tyrosine-protein phosphatase nonreceptor type 9; DUSP5/6, dual specificity phosphatase 5/6; SHP-2, SH2 domain-containing protein-tyrosine phosphatase-2; E2F1, E2F transcription factor 1; MEF2C, myocyte-specific enhancer-binding factor-2C; TOX, thymocyte selection associated high-mobility group box; Foxo1, forkhead box O1; Foxp3, forkhead box protein 3.

In addition, it was reported that miR-155 was involved in thymus development of iNKT cells. Burocchi et al. [43] used miR-155 overexpression in a mouse model and found that miR-155 overexpression could inhibit the development and maturation of iNKT cells in the thymus, showing that immature iNKT cells were largely retained in their second stage of thymus development ($CD24^{lo}CD44^{hi}NK1.1^{-}$), leading to a decrease in the number of iNKT cells migrating to the periphery. It was also found that the expression of 2 target molecules of miR-155, *Ets1* and *Itk*, was downregulated during iNKT cell maturation in this murine model [43]. However, the exact regulatory mechanism remains to be elucidated.

Other miRNAs

In addition to the above miRNA family molecules, other miRNA members also participate in the regulation of thymocyte development (Table 1). For instance, miR-142 is an evolutionarily conserved miRNA that can be selectively expressed in hematopoietic tissue. Mildner et al. [44] showed that the deletion of miR-142 could affect cell homeostasis. Their further analysis revealed that miR-142 was required for thymocyte precursor development and T-cell maturation. The lack of miR-142 could lead to the abnormal development of stasis of the thymocyte precursor, and impair thymocyte proliferation and differentiation, which result in a large number of cells being retained in the DP stage. For other examples, the expression level of miR-125b in lymphatic stem cells was higher than that of myeloid and hematopoietic stem cells. This high expression promotes the development of lymphocyte lineages and participates in the survival of hematopoietic stem cells and the maintenance of lymphoid balance [45]. miR-205 could promote the development of T cells after stress by regulating forkhead box N1 and specific chemokines [46]. Moreover, miR-223 could further regulate the development of T cells by protooncogene control [47]. In addition, miR-133b regulated the differentiation of NKT17 cells in the thymus by regulating Th-POK expression and dendritic cell signaling [48]. Most recently, we used miR-126 knockdown in mice and found that miR-126 deficiency could affect the development of thymic $CD4^{+}SP$ cells through elevating its target molecule *IRS-1* [49]. These studies further demonstrated the complexity of miRNA family molecules involved in thymocyte development.

Conclusions

In recent years, a series of studies have shown that miRNAs played an important regulatory role in thymocyte development, involving the development of lym-

phoid progenitor cells in the bone marrow, as well as DN/DP, and positive and negative selection of thymocytes. However, there are still many scientific issues to be further clarified. For example, what is the exact relationship among these different miRNA molecules, different stages of thymocyte development, and microenvironment in thymus? What about the related molecular mechanisms? The regulation mechanism of the expression of these miRNA molecules, especially the intrinsic link between the mRNAs involved in thymocyte development and clinical diseases, and how to carry out immunological biotherapy based on miRNA molecule-related clinical diseases? And so on. Further elucidation of these scientific issues will not only greatly enhance the understanding of the biological functions of miRNAs and the regulation mechanism of thymocyte development, but also be helpful in developing new strategies for immunotherapy against related clinical diseases.

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Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Lin Hu designed and wrote the paper; Ling Mao and Shiming Liu wrote the paper; Juanjuan Zhao, Chao Chen, Mengmeng Guo, and Jie Yang designed the paper; Lin Xu, Wei Xu, and Zhixu He conceived, designed, and wrote the paper; and all authors reviewed the paper.

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