Review Article

MicroRNAs Regulate Thymic Epithelium in Age-Related Thymic Involution via Down- or Upregulation of Transcription Factors

Minwen Xu,¹ Xiaoli Zhang,² Ruiyun Hong,¹ Dong-Ming Su,³ and Liefeng Wang²,³

¹First Affiliated Hospital, Gannan Medical University, Ganzhou 341000, China
²Department of Biotechnology, Gannan Medical University, Ganzhou 341000, China
³Institute for Molecular Medicine, University of North Texas Health Science Center, Fort Worth, TX 76107, USA

Correspondence should be addressed to Dong-Ming Su; dongming.su@unthsc.edu and Liefeng Wang; 469730795@qq.com

Received 19 April 2017; Revised 9 August 2017; Accepted 20 August 2017; Published 10 September 2017

Academic Editor: Luca Gattinoni

Copyright © 2017 Minwen Xu et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Age-related thymic involution is primarily induced by defects in nonhematopoietic thymic epithelial cells (TECs). It is characterized by dysfunction of multiple transcription factors (TFs), such as p63 and FoxN1, and also involves other TEC-associated regulators, such as Aire. These TFs and regulators are controlled by complicated regulatory networks, in which microRNAs (miRNAs) act as a key player. miRNAs can either directly target the 3′-UTRs (untranslated regions) of the TFs to suppress TF expression or target TF inhibitors to reduce or increase TF inhibitor expression and thereby indirectly enhance or inhibit TF expression. Here, we review the current understanding and recent studies about how miRNAs are involved in age-related thymic involution via regulation of TEC-autonomous TFs. We also discuss potential strategies for targeting miRNAs to rejuvenate age-related declined thymic function.

1. Introduction

The ubiquitous and abundant existence of small noncoding microRNAs (miRNAs) in worms, plants, and animals play an important role in the regulation of gene expression, which primarily occurs at posttranscriptional levels via cleavage and/or translational repression of messenger RNAs (mRNAs) [1]. Ample evidence shows that miRNAs control a wide range of developmental and physiological pathways, including cell proliferation [2], differentiation [3], and apoptosis [4]. Thus, deregulation of miRNAs will cause certain developmental obstructions, deficiencies, and even the onset of diseases [5]. The miRNA regulation is also engaged in several aspects of thymic biology [6], which are critical for T lymphopoiesis. The entire process of thymus organogenesis, maturation, and age-related involution is tightly regulated by transcription factors (TFs) [7], which, in turn, could be regulated at posttranscriptional level by miRNA genes [8, 9]. The thymus is composed of mainly hematopoietic thymocytes and nonhematopoietic thymic epithelial cells (TECs). TECs play a key role in supporting thymocyte development and controlling thymic aging. Although thymocytes possess their own transcription factors (TFs) to control their autonomous activities, many thymic activities during thymic development and aging can be regulated by known TFs in TECs, such as the p63 and FoxN1 [10–13]. However, regulation of these TFs remains mysterious and there is limited evidence as to the mechanisms involved. Given that many miRNAs are expressed in the thymus with different expression profiles at different developmental stages, we have adequate reasons to infer that miRNAs can be responsible for the regulation of TFs which are involved in maintaining normal thymic microenvironment that supports T lymphocyte development and controls age-related thymic involution. In this review, we focus on recent research progress which helps to elucidate how miRNA genes regulate TEC homeostasis and aging by affecting TEC-specific TFs. This summary about miRNA-mediated regulation will provide us some new insights into the regulatory networks underlying the construction...
and maintenance of the thymic microenvironment during thymic aging and even provide potential strategies for rejuvenating the function of the aged thymus.

2. Thymic Stromal Cell Homeostasis, Thymic Aging, and Transcriptional Regulation

The thymus is one of the most important organs in animal life. It generates T lymphocytes and supports the cellular immune system involved in the activities of antitumor, antivirus, and anti-intracellular infection, as well as in the establishment of self-tolerance to prevent autoimmune diseases. The thymus is also one of the most active organs, as it undergoes organogenesis (cell migration, proliferation, and differentiation), development (proliferation, differentiation, and cell apoptosis), and age-related involution (cell senescence and apoptosis) [14]. The aging process in the thymus starts in early adolescent years, and the typical thymic aging phenotype is thymic involution [15, 16].

There are two progenitor cell types in the thymus, hematopoietic thymocytes and nonhematopoietic TECs [17]. They interact and regulate each other in thymic development, homeostasis, and aging. Both cell types undergo a stepwise or sequential developmental process [18, 19]. In principle, TECs play a primary role in constructing the three-dimensional thymic meshwork and maintain the thymic microenvironment to support T cell development. TEC development and homeostasis are critical for determining thymic organogenesis prenatally and also regulate thymic involution during aging [20, 21].

Age-related thymic involution does not only reduce the output of naïve T cells but also increase the release of self-reactive T cells from the thymus [22]. These age-related changes create the basis for many age-related diseases, such as immunosenescence, chronic inflammatory diseases, cardiovascular and neurodegenerative diseases, autoimmunity, and cancer. Age-related thymic involution appears to be a defect primarily associated with TECs [23]. TEC development and homeostasis are very meticulous processes controlled by complex regulatory networks during thymus organogenesis, homeostasis, and aging [24], which involved multiple signaling pathways and cellular interactions. Transcription factors FoxN1 and p63 are crucial for TEC development. In the thymus, FoxN1, which plays an important role in TEC survival and differentiation [25, 26], promotes differentiation of thymic epithelial progenitor cells into functional medullary thymic epithelial cells (mTECs) and cortical thymic epithelial cells (cTECs) during organogenesis [27, 28] and maintains postnatal TEC homeostasis [29, 30]. The transcription factor p63 plays a crucial role for the epithelial development in several tissues, such as thymus and epidermis [31], and is essential for the proliferative potential of thymic epithelial progenitor cells [31, 32]. There are two p63 isoforms: one containing an N-terminal transactivation domain, named TAp63, while the other lacking this domain is named ΔNp63. ΔNp63 and FoxN1 are both highly expressed in the fetal thymus [11, 33], but, in the adult thymus, both FoxN1+ and ΔNp63+ TECs are decreased with age [10, 34, 35]. So far, the mechanism underlying this decline is largely unknown.

Another very important transcription factor expressed in mTECs is the autoimmune regulator (Aire) gene; the expression of which is also declined with age [36, 37]. Although it is uncertain whether Aire functions to regulate the differentiation of immature TECs [38], its role in regulating clonal deletion of self-reactive T cells is definite [39, 40]. Although thousands of target genes induced by Aire have already been identified and well characterized, the regulation of Aire gene itself remains elusive. Recently, many regulators which might act upstream of Aire have been identified [41]. For example, a FoxN1-Cre-induced ablation of DGCR8, a component of the miRNA-specific microprocessor complex, eliminated Aire expression in TECs, implying a potential role of miRNA in the regulation of Aire gene, since DGCR8 participates in the primary miRNA to pre-miRNA processing [42, 43]. However, the specific miRNAs involved in Aire regulation and the mechanisms by which they modulate Aire expression need further investigation.

3. A Fine-Tuning Role of miRNAs in Thymic Epithelial Cell Homeostasis

The miRNAs are posttranscriptional regulators involved in transcriptional repression or enhancement. Notably, a single miRNA can regulate multiple genes and a single gene can be regulated by multiple miRNAs [44]. Gene expression can be turned on either by TFs or indirectly by downregulation of other suppressive genes [45]. Expression of TFs can be suppressed either by miRNAs at their 3′-UTRs or by other suppressive genes. The suppressive genes can also be regulated by miRNAs [46]. A diagram of this regulatory network is schematically shown in Figure 1. Therefore, miRNAs play a fine-tuning role by targeting miRNAs of both TFs (direct suppression) and TF suppressors (indirect enhancement) for cleavage, translational repression, or chromatin modification [47–49]. miRNAs function in a wide range of biological processes including developmental regulation [50–52], hematopoietic cell lineage determination [53–55], cellular proliferation and death/apoptosis [56–61], fat metabolism [62, 63], neuronal patterning in nematodes [64, 65], chemosensory neurons asymmetric expression [64, 66], and oncogenesis [67–70].

Since expression of miRNAs is tightly related to tissue differentiation stages [71] and miRNAs can function to prevent cell division and drive terminal differentiation [72], miRNAs are very likely to be involved in TEC differentiation-driven thymic development and thymic involution [73]. For a given gene, its expression could be directly suppressed by some miRNAs or activated indirectly via miRNA-mediated inhibition of its upstream suppressor (Figure 1). Therefore, a mixed miRNA pool, instead of a single miRNA, is more likely to orchestrate the regulatory network involved in thymic development and aging. Within a given miRNA pool, some miRNAs may suppress certain genes, while others may suppress
inhibitory genes to indirectly turn on the suppressed/silent genes. Therefore, the complicated and intricate regulatory network in the thymus can potentially be regulated for development and rejuvenation by a mixed miRNA pool, rather than by a single miRNA.

As expected, recent studies have demonstrated the role of miRNAs in TEC biology. Cortical TECs (cTECs), immature medullary TEClow (mTEClow), and mature mTEChigh cells were used for miRNA microarray analysis, which demonstrated that the miRNA expression profile changes as the cell matures [74]. When the entire miRNA pool was abolished in TECs by conditionally deleting Dicer, which is the miRNA maturation enzyme responsible for cleaving the pre-miRNA to the miRNA duplex, the apoptosis of mTECs was induced and cTECs failed for cleaving the pre-miRNA to the miRNA duplex, Dicer, which is the miRNA maturation enzyme responsible. As mentioned above, miR-205 plays an important role in supporting TEC differentiation in the fetal and adult thymus, and miRNAs can regulate TEC development and differentiation by directly or indirectly targeting FoxN1 gene (Figure 1). There are four reports providing evidence to confirm this point of view.

Firstly, using a miR-205Δ/Δ:FoxN1-Cre mice to delete miR-205 in all TECs in the thymus, Hoover group demonstrated that miR-205 plays an important role in supporting T cell development following high-dose inflammatory perturbations, because conditional ablation of miR-205 caused a severe thymic hypoplasia and delayed T cell recovery, accompanied with gene expression changes in chemokine/chemokine receptor pathways, antigen processing components, and WNT signaling system [76]. Hoover group also found that miR-205 is highly expressed in both cTECs and mTECs but is largely dispensable for thymus recovery in response to low-level inflammation [73, 77]. Compared to the miR-205Δ/Δ:FoxN1-Cre conditional knockout mice, FoxN1 expression levels were 2-fold higher in FoxN1Cre mice. This expression change was also confirmed using fetal thymic organ culture prepared from E14.5 (gestation at 14.5 days) embryos from wild type and miR-205Δ/Δ:FoxN1-Cre mice. The results suggest that miR-205 is required for FoxN1 expression and epithelial cell function in fetal organogenesis and adult homeostasis following inflammatory perturbations [76]. Furthermore, incubation with miR-205 mimics (called agonirs) restored FoxN1 levels in the fetal thymic organ culture model. MiR-205 agonirs also increased the levels of ccl25 and stem cell factor (SCF), which are downstream targets for FoxN1. MiR-205 regulates FoxN1 levels in TECs probably by promoting the degradation of miRNAs whose products suppress FoxN1 expression (diagramed in Figure 1, indirect impact). The authors tried to assess

**Figure 1:** miRNA fine-tune age-related thymic involution through regulation of TEC-autonomous transcription factors. (a) Under normal conditions, a given TF (such as Foxn1) is fine-tuned by miRNAs at its 3′-UTR sites; meanwhile, the TF is also potentially regulated by its suppressive factors, which is also fine-tuned at their 3′UTR sites by miRNAs. The regulatory networks coregulate TF expression; (b) in the aged condition, some miRNAs, which directly suppress TFs, are potentially increased (from + to ++). At the same time, other miRNAs, which suppress TF suppressors, may be decreased (from +++ to +), which results in enhancement of the TF-suppressor expression which inhibit TF expression. The consequence of this combination is that the TF level is decreased. If the TF for TEC homeostasis is decreased during aging, age-related thymic involution takes place.

**4. miRNAs Play a Role in Thymic Epithelial Cell Development and Homeostasis by Regulating Critical Transcriptional Factors**

As mentioned above, FoxN1 acts as a key regulator of TEC development and differentiation in the fetal and adult thymus, and miRNAs can regulate TEC development and differentiation by directly or indirectly targeting FoxN1 gene (Figure 1). There are four reports providing evidence to confirm this point of view.

Firstly, using a miR-205Δ/Δ:FoxN1-Cre mice to delete miR-205 in all TECs in the thymus, Hoover group demonstrated that miR-205 plays an important role in supporting T cell development following high-dose inflammatory perturbations, because conditional ablation of miR-205 caused a severe thymic hypoplasia and delayed T cell recovery, accompanied with gene expression changes in chemokine/chemokine receptor pathways, antigen processing components, and WNT signaling system [76]. Hoover group also found that miR-205 is highly expressed in both cTECs and mTECs but is largely dispensable for thymus recovery in response to low-level inflammation [73, 77]. Compared to the miR-205Δ/Δ:FoxN1-Cre conditional knockout mice, FoxN1 expression levels were 2-fold higher in FoxN1Cre mice. This expression change was also confirmed using fetal thymic organ culture prepared from E14.5 (gestation at 14.5 days) embryos from wild type and miR-205Δ/Δ:FoxN1-Cre mice. The results suggest that miR-205 is required for FoxN1 expression and epithelial cell function in fetal organogenesis and adult homeostasis following inflammatory perturbations [76]. Furthermore, incubation with miR-205 mimics (called agonirs) restored FoxN1 levels in the fetal thymic organ culture model. MiR-205 agonirs also increased the levels of ccl25 and stem cell factor (SCF), which are downstream targets for FoxN1. MiR-205 regulates FoxN1 levels in TECs probably by promoting the degradation of miRNAs whose products suppress FoxN1 expression (diagramed in Figure 1, indirect impact). The authors tried to assess
whether 3′-UTRs in any of nineteen candidate genes had 3 or more predicted miR-205 binding sites, in order to find genes that impact FoxN1 [76]. In addition to miR-205, miR-18b and miR-518b were also found to affect FoxN1 by suppressing its expression, potentially through directly targeting FoxN1 3′-UTRs (diagramed in Figure 1, direct impact).

In the second approach, Kushwaha et al. performed miRNA profiling of bone morphogenetic protein-2-treated NT2/D1 cells using the Agilent Human V2 miRNA v.10.1 array and screened out two miRNAs, miR-18b and miR-518b, which directly bind to FoxN1 3′-UTRs and inhibit FoxN1 expression [78]. Interfering with these two miRNAs separately or simultaneously can increase FoxN1 gene expression. When these two miRNAs were overexpressed separately or simultaneously, FoxN1 expression was down-regulated. These results demonstrate that miR-18b and miR-518b are upstream controllers of FoxN1 in TECs [78]. Thirdly, miR-22 is also a posttranscriptional regulator which directly represses FoxN1 [9]. In a TRE-miR-22 mouse model (K14-rTA/TRE-miR-22 double transgenic mice), miR-22 overexpression in the skin promoted the anagen-to-catagen transition, inhibited keratinocyte expansion and differentiation, and enhanced hair follicle apoptosis. Since hair development is regulated by multiple hair differentiation regulators, including Dlx3, Hoxc13, FoxN1, and Lef1, miR-22 potentially directly targets these genes [9]. Given that miR-22 impacts epithelial cell development in the skin and might regulate FoxN1, a logical assumption is that miR-22 is likely to control the function of thymic epithelial cells. Finally, there was a recent report in which miR125a-5p, whose expression is increased in the aged thymus, was found to negatively regulate FoxN1 expression in the aged thymus [79].

Transcription factor Tp63, a homolog of the tumor suppressor p53, is critical for the development of epithelial tissues, including the thymus [80]. The p63-FoxN1 regulatory axis has been shown to regulate postnatal TEC homeostasis in Su group’s work [10], but the study failed to identify the upstream effector responsible for regulating this axis. It has been reported that a number of miRNAs play an important role in epidermal cell proliferation and homeostasis by targeting p63 [81–84], implying that these miRNAs may play a role in thymic development.

The p63 gene functions as an essential regulator of stem cell maintenance in stratified epithelial tissues and is also a target of some miRNAs. For example, miR-203 has an immediate and long-term impact on epidermal cell proliferation by directly regulating p63 [85–88]. MiR-203 was reported to promote epidermal differentiation by restricting proliferative potential and inducing cell cycle exit through directly repressing p63 [88]. To support that, Jackson group used established keratinocytes from K14-rTA/pTRE2-miR-203 double positive skin and found that miR-203 is closely correlated with the epidermal differentiation in a spatiotemporally specific manner by both immediate inhibition of cell cycle progression and long-term inhibition of stem cell self-renewal [85]. They also identified a pool of miR-203-targeted genes using a genome-wide approach. These miR-203-targeted genes, including p63, Msi2, and Skp2, play a coregulatory role that is crucial for driving cell cycle exit and restricting proliferative potential [85]. Furthermore, Chikh et al. demonstrated that the inhibitory apoptosis-stimulating protein of p53 (iASPP), a member of the apoptosis-stimulating protein of p53 (ASPP) family, represses p63 expression through miR-574-3p and miR-720. They found that iASPP is required for the homeostasis of epithelia [89]. MiR-720 and miR-574-3p were found to be upregulated as a consequence of iASPP silencing using an Agilent microRNA profiling assay. When coexpressed with a luciferase reporter gene containing the 3′-UTR of human p63, both MiR-720 and miR-574-3p significantly reduced luciferase activity. Use of antagonirs for miR-574-3p and miR-720 in keratinocytes restored ∆Np63 endogenous protein levels in sh-iASPP cells. Furthermore, using antagonirs for miR-574-3p and miR-720 can both prevent the ∆Np63 down-regulation typically observed during primary keratinocyte differentiation [89]. In addition, miR-130b has been reported to directly repress ∆Np63 expression in keratinocyte senescence [84].

On the other hand, p63 can regulate the expression of some miRNAs. TAp63 binds to and transactivates the Dicer promoter and suppresses metastasis through the regulation of Dicer and a number of specific miRNAs, including miR-130b [90]. ∆Np63 in epithelial cells is a transcriptional regulator of DGC88, which localizes to the cell nucleus and is required for miRNA processing [91]. Further, p63 mediated cell cycle progression in epidermal cells by directly repressing miR-34a and miR-34c [92]. Many miRNAs, such as miR-192/215, miR-107, miR-96,132, and miR-145, are known transcriptional targets of p63 [46, 93]. Wu group has elucidated multiple p63-regulated miRNAs’ (miR-17, miR-20b, miR-30a, miR-106a, miR-143, and miR-455-3p) roles in the onset of keratinocyte differentiation [81]. It should be noted that all these experiments were conducted in skin epithelial cells, and therefore no direct evidence has been found yet to show that miRNA regulation on p63 is also engaged in thymic development and aging. Although skin epithelial cells share many similarities with TECs and these findings can provide a shortcut to study miRNA regulation in TECs, subsequent experiments in TECs are still required.

Aire gene is a transcription factor that controls expression of peripheral tissue antigen (PTA) genes in mTECs. Aire controls hundreds or even thousands of PTAs and has been proposed to function as a nonclassical TF based on the fact that the gene does not have many DNA-binding sites for direct interaction [94]. As for the regulation of Aire, specific miRNAs, such as miR-29a, in TECs play a key role. Deletion of miR-29a resulted in a progressively decreased expression of Aire and Aire-dependent genes in a miR-29a null mutant mouse model [74]. Additionally, miR-220b may act as a regulator for Aire gene translation, since mutation in miR-220R significantly reduced the level of Aire protein [95]. Although there is insufficient evidence that Aire expression is regulated by miRNAs, Aire has been shown to control 30 Aire-dependent miRNAs. Eighteen of these 30 miRNAs were
upregulated, and the rest were downregulated in Aire-silenced thymic mTECs [96], strongly suggesting that these miRNAs are under the control of Aire. Therefore, Aire might function as an upstream controller of these miRNAs, which in turn, plays a potential role in the control of PTAs in mTECs [42, 74, 96, 97]. Microarray profiling of TEC subpopulations showed that series of miRNAs were significantly upregulated during terminal mTEC differentiation. For example, miR-124, miR-129, miR-202, miR-203, miR-302b, and miR-467a were expressed at two- to tenfold higher levels in the mTEC high than in the mTEC low (expression levels were all normalized to MHC-II surface expression levels) both in mouse and human thymus. The mTEC high population can be further divided into Aire+ and Aire− subsets, and the above-mentioned miRNAs were all downregulated in Aire−mTEC high compared to Aire+mTEC high, with the exception of miR-302b, suggesting a mutual regulatory relationship between Aire and miRNAs during mTEC maturation. It was further demonstrated that miR-202 was upregulated in both immature and mature mTECs of Aire null mutants, while miR-129, miR-499, and miR-302b were significantly downregulated in mature mTECs of Aire null mutants compared to wild type mice [74]. To determine which miRNA controls PTAs in the mTECs and whether Aire expression levels could affect these interactions, Oliveira group constructed miRNA-mRNA interaction networks and found that miRNA let-7b interacted with the PTA mRNAs and confirmed the existence of a link between Aire and miRNAs in controlling the promiscuous gene expression pattern in mTECs [94].

5. Potential Strategies to Rejuvenate Age-Related Declined Thymic Function by Targeting miRNAs with Agomirs and Inhibitors

Although the mechanism of thymic involution has not been fully understood yet, the role played by miRNAs in this process cannot be ignored [98, 99]. For example, Guo group demonstrated that miR-181a-5p expression was increased in aged TECs, which might contribute to age-related thymic involution through downregulating the phosphorylation of Smad3 and blocking the activation of the TGF-β signaling [98]. WNT signaling in thymic epithelia is essential for normal thymus development and function [100] and was suppressed in the senescent human thymus [99]. Studies compared the difference in miRNA expression between old (70-year-old men) and young (<10-month-old newborns) thymus and found that miRNAs, such as miR-25, miR-7f, and miR-134, which are known modulators of the WNT pathway, were also altered [99]. Since TEC development and homeostasis are mostly controlled by p63, FoxN1, and Aire, miRNAs associated with these genes would be potential targets of therapeutic value. Targeting miRNAs with mimics or inhibitors is a potential strategy to rejuvenate age-related declined thymic function. In one of our published reports, we found that miRNA pools from young and aged thymus have different spectrums [79]. The strategy to rejuvenate age-related declined thymic function would be to suppress upregulated miRNAs and promote downregulated miRNAs in the senescent TECs. We hypothesize that a mixed pool of miRNA is involved in the regulation of age-related thymic involution. Therefore, multiple combinations of synthesized miRNA mimics (agomirs) targeting the downregulated miRNAs and miRNA inhibitors (antagomirs) against the upregulated miRNAs are probably the best solution to restore the age-related declined thymic function.

Thymic atrophy is attributed to increased age-related chronic inflammation, and suppressing this inflammation may alleviate thymic atrophy or restore thymic function [101]. Since miRNAs also control inflammation reactions, this might provide another approach to rejuvenating age-related thymic involution. For example, miR146a was reported to suppress inflammation, miR155 was reported to promote inflammation, and the absence of miR146a [34, 102], or upregulation of miR155 [103–105], promotes chronic inflammation with age. Furthermore, miR146a and miR155 counterregulate the immune response during chronic inflammation. Thus, combinational application of miR146a-agonir and miR155-antagonir might attenuate age-related atrophied thymic inflammation, thereby improving central immune tolerance generation.

6. Summary

In conclusion, miRNAs play a role in fine-tuning multiple transcription factor (TF) expression in TECs and thereby have a significant impact on thymus organogenesis, maturation, and involution at a posttranscriptional level. We reviewed recent progresses in studying the potential function of miRNAs in age-related thymic involution. Apparently, TEC development, homeostasis, and involution are very complicated processes each with a comprehensive regulatory network. Without a doubt, transcription factors p63, FoxN1, and Aire should be the primary targets for rejuvenating age-related declined thymic function. Modulation of the miRNA levels for regulating these TFs in the aged thymus via synthesized miRNA mimics (agomirs) or miRNA inhibitors (antagomirs) might provide an efficient approach for rejuvenating age-related thymic involution. Although current evidence is still insufficient for explaining how miRNAs regulate these TEC-autonomous TFs and subsequently induce thymic involution, we hope this review will help to summarize previous studies and guide future work towards discovering potential miRNA candidates for therapeutic targets.

Abbreviations

miRNA: MicroRNA
TEC: Thymic epithelial cell
cTEC: Cortical thymic epithelial cell
mTEC: Medullary thymic epithelial cell
TF: Transcription factors
Aire: Autoimmune regulator
UTR: Untranslated region
ASPP: Apoptosis-stimulating protein of p53
iASPP: Inhibitory of ASPP
PTA: Peripheral tissue antigen.
Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

This work was partially supported by grants from the Higher Education Foundation of Jiangxi Provincial (KJLD2090), the National Science Foundation of Jiangxi Province (20132BAB205032), and the National Natural Science Foundation of China (31260279 and 31660256) to Liefeng Wang.

References

[1] C. Z. Chen, L. Li, H. F. Lodish, and D. P. Bartel, "MicroRNAs modulate hematopoietic lineage differentiation," Science, vol. 303, no. 5654, pp. 83–86, 2004.
[2] S. Kohlihaas, O. A. Garden, C. Scudamore, M. Turner, K. Okkenhaug, and E. Vigorito, "Cutting edge: the Foxp3 target miR-155 contributes to the development of regulatory T cells," Journal of Immunology, vol. 182, no. 5, pp. 2578–2582, 2009.
[3] S. A. Muljo, K. M. Ansel, C. Kanellopoulou, D. M. Livingston, A. Rao, and K. Rajewsky, "Aberant T cell differentiation in the absence of Dicer," The Journal of Experimental Medicine, vol. 202, no. 2, pp. 261–269, 2005.
[4] L. Deng, H. Liang, M. Xu et al., "STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors," Immunity, vol. 41, no. 5, pp. 843–852, 2014.
[5] H. X. Chu, H. A. Kim, S. Lee et al., "Immune cell infiltration in malignant middle cerebral artery infarction: comparison with transient cerebral ischemia," Journal of Cerebral Blood Flow and Metabolism, vol. 34, no. 3, pp. 450–459, 2014.
[6] K. S. Kang and J. E. Trosko, "Stem cells in toxicology: fundamental biology and practical considerations," Toxicological Sciences, vol. 120, Supplement 1, pp. S269–S289, 2011.
[7] P. M. Garfin, D. Min, J. L. Bryson et al., "Inactivation of the RB family prevents thymus involution and promotes thymic function by direct control of Foxn1 expression," The Journal of Experimental Medicine, vol. 210, no. 6, pp. 1087–1097, 2013.
[8] J. B. Tagne, O. R. Mohtar, J. D. Campbell et al., "Transcription factor and microRNA interactions in lung cells: an inhibitory link between NK2 homeobox 1, miR-200c and the developmental and oncogenic factors Nfib and Myb," Respiratory Research, vol. 16, p. 22, 2015.
[9] S. Yuan, F. Li, Q. Meng et al., "Post-transcriptional regulation of keratinocyte progenitor cell expansion, differentiation and hair follicle regression by miR-22," PLoS Genetics, vol. 11, no. 5, article e1005253, 2015.
[10] P. Burnley, M. Rahman, H. Wang et al., "Role of the p63-Foxn1 regulatory axis in thymic epithelial cell homeostasis during aging," Cell Death & Disease, vol. 4, article e932, 2013.
[11] E. Candi, A. Rufini, A. Terrinoni et al., "DeltaNp63 regulates thymic development through enhanced expression of FgfR2 and Jag2," Proceedings of the National Academy of Sciences of the United States of America, vol. 104, no. 29, pp. 11999–12004, 2007.
[12] R. V. Chilukuri, V. K. Patel, M. Martinez, J. C. Guyden, and M. D. Samms, "The antigenic determinant that defines thymic nurse cells is expressed by thymic epithelial progenitor cells," Frontiers in Cell and Development Biology, vol. 2, no. 13, 2014.
[13] R. Romano, L. Palamaro, A. Fusco et al., "FOXN1: a master regulator gene of thymic epithelial development program," Frontiers in Immunology, vol. 4, p. 187, 2013.
[14] O. Gressner, T. Schilling, K. Lorenz et al., "TAp63alpha induces apoptosis by activating signaling via death receptors and mitochondria," The EMBO Journal, vol. 24, no. 13, pp. 2458–2471, 2005.
[15] D. D. Taub and D. L. Longo, "Insights into thymic aging and regeneration," Immunological Reviews, vol. 205, pp. 72–93, 2005.
[16] H. E. Lynch, G. L. Goldberg, A. Chidgey, M. R. Van den Brink, R. Boyd, and G. D. Sempowski, "Thymic involution and immune reconstitution," Trends in Immunology, vol. 30, no. 7, pp. 366–373, 2009.
[17] J. Abramson and G. Anderson, "Thymic epithelial cells," Annual Review of Immunology, vol. 35, pp. 85–118, 2017.
[18] D. B. Klug, C. Carter, E. Crouch, D. Roop, C. J. Conti, and E. R. Richie, "Interdependence of cortical thymic epithelial cell differentiation and T-lineage commitment," Proceedings of the National Academy of Sciences of the United States of America, vol. 95, no. 20, pp. 11822–11827, 1998.
[19] W. van Ewijk, G. Hollander, C. Terhorst, and B. Wang, "Stepwise development of thymic microenvironments in vivo is regulated by thymocyte subsets," Development, vol. 127, no. 8, pp. 1583–1591, 2000.
[20] X. Zhu, J. Gui, J. Dohkan, L. Cheng, P. F. Barnes, and D. M. Su, "Lymphohematopoietic progenitors do not have a synchronized defect with age-related thymic involution," Aging Cell, vol. 6, no. 5, pp. 663–672, 2007.
[21] D. M. Su, D. Aw, and D. B. Palmer, "Immunosenescence: a product of the environment?" Current Opinion in Immunology, vol. 25, no. 4, pp. 498–503, 2013.
[22] B. Coder and D. M. Su, "Thymic involution beyond T-cell insufficiency," Oncotarget, vol. 6, no. 26, pp. 21777-21778, 2015.
[23] L. Sun, J. Guo, R. Brown, T. Amagai, Y. Zhao, and D. M. Su, "Declining expression of a single epithelial cell-autonomous gene accelerates age-related thymic involution," Aging Cell, vol. 9, no. 3, pp. 347–357, 2010.
[24] Y. Takahama, I. Ohigashi, S. Baik, and G. Anderson, "Generation of diversity in thymic epithelial cells," Nature Reviews Immunology, vol. 17, no. 5, pp. 295–305, 2017.
[25] M. Itoi, H. Kawamoto, Y. Katsura, and T. Amagai, "Two distinct steps of immigration of hematopoietic progenitors into the early thymus anlage," International Immunology, vol. 13, no. 9, pp. 1203–1211, 2001.
[26] C. Chen, Y. Liu, and P. Zheng, "mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells," Science Signaling, vol. 2, no. 98, article ra75, 2009.
[27] M. Nehls, K. Luno, M. Schorpp et al., "A yeast artificial chromosome contig on mouse chromosome 11 encompassing the nu locus," European Journal of Immunology, vol. 24, no. 7, pp. 1721–1723, 1994.
[28] D. Lee, D. M. Prowse, and J. L. Brissette, "Association between mouse nude gene expression and the initiation of
epithelial terminal differentiation,” Developmental Biology, vol. 208, no. 2, pp. 362–374, 1999.

[29] L. Cheng, J. Guo, L. Sun et al., “Postnatal tissue-specific disruption of transcription factor Foxn1 triggers acute thymic atrophy,” The Journal of Biological Chemistry, vol. 285, no. 8, pp. 5836–5847, 2010.

[30] L. Chen, S. Xiao, and N. R. Manley, “Foxn1 is required to maintain the postnatal thymic microenvironment in a dosage-sensitive manner,” Blood, vol. 113, no. 3, pp. 567–574, 2009.

[31] A. S. Adler, T. L. Kawahara, E. Segal, and H. Y. Chang, “One domain of Foxn1 required for crosstalk-dependent thymic epithelial cell differentiation,” Nature Immunology, vol. 4, no. 11, pp. 1128–1135, 2003.

[32] L. Cheng, J. Guo, L. Sun et al., “Aire protein, a thymus resident molecule, is associated with cellular migration, proliferation and apoptosis in human breast cancer cells,” Journal of Immunology Research, vol. 2016, pp. 604–611, 2015.

[33] A. S. Papadopoulou, J. Dooley, M. A. Linterman et al., “The thymic epithelial microRNA network elevates the threshold for infection-associated thymic involution via miR-29a mediated suppression of the IFN-alpha receptor,” Nature Immunology, vol. 13, no. 2, pp. 181–187, 2011.

[34] I. S. Khan, R. T. Taniguchi, K. J. Fasano, M. S. Anderson, and L. T. Jeker, “Canonical microRNAs in thymic epithelial cells promote central tolerance,” European Journal of Immunology, vol. 44, no. 5, pp. 1313–1319, 2014.

[35] J. Gordon, A. R. Bennett, C. C. Blackburn, and N. R. Manley, “Gcm2 and Foxn1 mark early parathyroid- and thymus-specific domains in the developing thymic pouch,” Mechanisms of Development, vol. 103, no. 1-2, pp. 141–143, 2001.

[36] L. Boominathan, “The tumor suppressors p53, p63, and p73 are regulators of microRNA processing complex,” PLoS One, vol. 5, no. 5, article e10615, 2010.

[37] D. P. Bartel, “MicroRNAs: genomics, biogenesis, mechanism, and function,” Cell, vol. 116, no. 2, pp. 281–297, 2004.

[38] G. Pepin and M. P. Gantier, “microRNA decay: refining microRNA regulatory activity,” MicroRNA, vol. 5, no. 3, pp. 167–174, 2016.

[39] L. T. Jeker, “Re-expression of microRNA-150 induces EBV-positive Burkitt lymphoma differentiation by modulating c-Myb in vitro,” Cancer Science, vol. 104, no. 7, pp. 826–834, 2013.

[40] T. Chen, A. Margariti, S. Kelaini et al., “MicroRNA-199b modulates vascular cell fate during iPSC cell differentiation by targeting the notch ligand Jagged1 and enhancing VEGF signaling,” Stem Cells, vol. 33, no. 5, pp. 1405–1418, 2015.

[41] R. W. Georgantas 3rd, R. Hildreth, S. Morisot et al., “CD34+ hematopoietic stem-progenitor cell microRNA expression and function: a circuit diagram of differentiation control,” Proceedings of the National Academy of Sciences of the United States of America, vol. 104, no. 8, pp. 2750–2755, 2007.

[42] M. S. Anderson, E. S. Venanzi, L. Klein et al., “Projection of an immunological self shadow within the thymus by the aire protein,” Science, vol. 298, no. 5597, pp. 1395–1401, 2002.

[43] Y. Herzig, S. Nevo, C. Bornstein et al., “Transcriptional programs that control expression of the autoimmune regulator gene Aire,” Nature Immunology, vol. 18, no. 2, pp. 161–172, 2017.

[44] A. S. Papadopoulou, J. Dooley, M. A. Linterman et al., “The thymic epithelial microRNA network elevates the threshold for infection-associated thymic involution via miR-29a mediated suppression of the IFN-alpha receptor,” Nature Immunology, vol. 13, no. 2, pp. 181–187, 2011.

[45] I. S. Khan, R. T. Taniguchi, K. J. Fasano, M. S. Anderson, and L. T. Jeker, “Canonical microRNAs in thymic epithelial cells promote central tolerance,” European Journal of Immunology, vol. 44, no. 5, pp. 1313–1319, 2014.

[46] G. A. Passos, D. A. Mendes-da-Cruz, and E. H. Oliveira, “The thymic orchestration involving Aire, microRNAs, and cell-cell interactions during the induction of central tolerance,” Frontiers in Immunology, vol. 6, p. 352, 2015.

[47] J. Gordon, A. R. Bennett, C. C. Blackburn, and N. R. Manley, “Gcm2 and Foxn1 mark early parathyroid- and thymus-specific domains in the developing third pharyngeal pouch,” Mechanisms of Development, vol. 103, no. 1-2, pp. 141–143, 2001.
mechanisms downstream of the oncogene KSHV-vGPCR, "instability by a broad suppression of genome maintenance"

A. Meerson, M. Traurig, V. Ossowski, J. M. Fleming, M. I. S. Khan, C. Y. Park, A. Mavropoulos et al., "miR-205 as a microRNA that is highly expressed in right neuronal asymmetry in Caenorhabditis elegans," vol. 426, no. 6968, pp. 845–849, 2015.

S. Chang, R. J. Johnston Jr., C. Frokjaer-Jensen, S. Lockery, and O. Hobert, "MicroRNAs act sequentially and asymmetrically to control chemosensory laterality in the nematode," Nature, vol. 426, no. 7001, pp. 785–789, 2004.

L. Sun, Q. Wang, X. Gao, D. Shi, S. Mi, and Q. Han, "miR-454 functions as an oncogene by regulating PTEN in uveal melanoma," FEBS Letters, vol. 589, no. 19 Part B, pp. 2791–2796, 2015.

C. J. Krause, O. Popp, N. Thirunarayanan, G. Dittmar, M. Lipp, and G. Muller, "MicroRNA-34a promotes genomic instability by a broad suppression of genome maintenance mechanisms downstream of the oncogene KSHV-vGPCR," Oncotarget, vol. 7, no. 9, pp. 10414–10432, 2016.

M. J. Bueno, I. Pérez de Castro, M. Gómez de Cedrón et al., "Genetic and epigenetic silencing of MicroRNA-203 enhances ABL1 and BCR-ABL1 oncogene expression," Cancer Cell, vol. 29, no. 4, pp. 607–608, 2016.

I. Fukumoto, K. Koshizuka, T. Hanazawa et al., "The tumor-suppressive microRNA-23b/27b cluster regulates the MET oncogene in oral squamous cell carcinoma," International Journal of Oncology, vol. 49, no. 3, pp. 1119–1129, 2016.

J. Lu, G. Getz, E. A. Miska et al., "MicroRNA expression profiles classify human cancers," Nature, vol. 435, no. 7043, pp. 834–838, 2005.

B. J. Reinhart, F. J. Slack, M. Basson et al., "The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans," Nature, vol. 403, no. 6772, pp. 901–906, 2000.

I. S. Khan, C. Y. Park, A. Mavropoulos et al., "Identification of MiR-205 as a microRNA that is highly expressed in medullary thymic epithelial cells," PLoS One, vol. 10, no. 8, article e0135440, 2015.

O. Ucar, L. O. Tykocinski, J. Dooley, A. Liston, and B. Kyewski, "An evolutionarily conserved mutual interdependence between Aire and microRNAs in promiscuous gene expression," European Journal of Immunology, vol. 43, no. 7, pp. 1769–1778, 2013.

S. Zuklys, C. E. Mayer, S. Zhanbekteva et al., "MicroRNAs control the maintenance of thymic epithelia and their competence for T lineage commitment and thymocyte selection," Journal of Immunology, vol. 189, no. 8, pp. 3894–3904, 2012.

A. R. Hoover, I. Dozmorov, J. MacLeod et al., "MicroRNA-205 maintains T cell development following stress by regulating forkhead box N1 and selected chemokines," The Journal of Biological Chemistry, vol. 291, no. 44, pp. 23237–23247, 2016.
stratified epithelia," *The EMBO Journal*, vol. 30, no. 20, pp. 4261–4273, 2011.

[90] X. Su, D. Chakravarti, M. S. Cho et al., "TAp63 suppresses metastasis through coordinate regulation of Dicer and miRNAs," *Nature*, vol. 467, no. 7318, pp. 986–990, 2010.

[91] D. Chakravarti, X. Su, M. S. Cho et al., "Induced multipotency in adult keratinocytes through down-regulation of DeltaNp63 or DGCR8," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 5, pp. E572–E581, 2014.

[92] D. Antonini, M. T. Russo, L. De Rosa, M. Gorrese, L. Del Vecchio, and C. Missero, "Transcriptional repression of miR-34 family contributes to p63-mediated cell cycle progression in epidermal cells," *The Journal of Investigative Dermatology*, vol. 130, no. 5, pp. 1249–1257, 2010.

[93] L. Boominathan, "The guardians of the genome (p53, TA-p73, and TA-p63) are regulators of tumor suppressor miRNAs network," *Cancer Metastasis Reviews*, vol. 29, no. 4, pp. 613–639, 2010.

[94] E. H. Oliveira, C. Macedo, C. V. Collares et al., "Aire down-regulation is associated with changes in the posttranscriptional control of peripheral tissue antigens in medullary thymic epithelial cells," *Frontiers in Immunology*, vol. 7, p. 526, 2016.

[95] T. Matsuo, Y. Noguchi, M. Shindo et al., "Regulation of human autoimmune regulator (AIRE) gene translation by miR-220b," *Gene*, vol. 530, no. 1, pp. 19–25, 2013.

[96] C. Macedo, A. F. Evangelista, M. M. Marques et al., "Autoimmune regulator (Aire) controls the expression of microRNAs in medullary thymic epithelial cells," *Immunobiology*, vol. 218, no. 4, pp. 554–560, 2013.

[97] G. A. Passos, D. A. Mendes-da-Cruz, and E. H. Oliveira, "Editorial: the role of Aire, microRNAs and cell-cell interactions on thymic architecture and induction of tolerance," *Frontiers in Immunology*, vol. 6, p. 615, 2015.

[98] D. Guo, Y. Ye, J. Qi et al., "MicroRNA-181a-5p enhances cell proliferation in medullary thymic epithelial cells via regulating TGF-β signaling," *Acta Biochimica Biophysica Sinica (Shanghai)*, vol. 48, no. 9, pp. 840–849, 2016.

[99] S. Ferrando-Martinez, E. Ruiz-Mateos, J. A. Dudakov et al., "WNT signaling suppression in the senescent human thymus," *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, vol. 70, no. 3, pp. 273–281, 2015.

[100] S. Zuklys, J. Gill, M. P. Keller et al., "Stabilized beta-catenin in thymic epithelial cells blocks thymus development and function," *Journal of Immunology*, vol. 182, no. 5, pp. 2997–3007, 2009.

[101] G. D. Sempowski, L. P. Hale, J. S. Sundy et al., "Leukemia inhibitory factor, oncostatin M, IL-6, and stem cell factor mRNA expression in human thymus increases with age and is associated with thymic atrophy," *Journal of Immunology*, vol. 164, no. 4, pp. 2180–2187, 2000.

[102] J. L. Zhao, D. S. Rao, M. P. Boldin, K. D. Taganov, R. M. O’Connell, and D. Baltimore, "NF-kappaB dysregulation in microRNA-146a-deficient mice drives the development of myeloid malignancies," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 22, pp. 9184–9189, 2011.

[103] R. M. O’Connell, D. Kahn, W. S. Gibson et al., "MicroRNA-155 promotes autoimmune inflammation by enhancing inflammatory T cell development," *Immunity*, vol. 33, no. 4, pp. 607–619, 2010.

[104] R. Hu, D. A. Kagele, T. B. Huffaker et al., "miR-155 promotes T follicular helper cell accumulation during chronic, low-grade inflammation," *Immunity*, vol. 41, no. 4, pp. 605–619, 2014.

[105] M. Nazari-Jahantigh, Y. Wei, H. Noels et al., "MicroRNA-155 promotes atherosclerosis by repressing Bcl6 in macrophages," *The Journal of Clinical Investigation*, vol. 122, no. 11, pp. 4190–4202, 2012.