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Effect of aging on microRNAs and regulation of pathogen recognition receptors

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Abstract

Immunosenescence is the multifactorial age-associated immune deterioration that leads to increased susceptibility to infections and decreased responses to vaccines. Recent studies have shown a fundamental role for microRNAs (miRNAs) in regulating immune responses, and nearly all the miRNAs involved in immune regulation show modulation during aging. Aging-associated miRNAs are largely negative regulators of the immune innate response and target central nodes of aging-associated networks, in particular, NF- κ B, the downstream effector of TLR signals that leads to induction of proinflammatory responses. Multiple miRNAs have been reported to share similar regulatory activity. Here we review miRNA regulation of human innate immune recognition in aging, including both activation and resolution of inflammation, critical issues in detection, and areas of active investigation into our understanding of immunosenescence.

Introduction

Immunosenescence is the multifactorial age-associated immune deterioration that leads to increased susceptibility to viral and bacterial infections and decreased responses to vaccines. Well documented functional impairments in lymphocyte development and signalling diminish adaptive immune responses in aging [1–3]. The age-related reduced responses in innate immune pathways are less well understood and paradoxically are accompanied by an elevation of basal pro-inflammatory signalling [3, 4]. Recent studies have identified non-coding microRNAs (miRNAs), which are typically 21–23 nucleotides sequences encoded by DNA, and which have broad functions in regulation of gene expression [5]. Although miRNAs are a relatively new research area, their role in aging processes has already shed light on how organism lifespan, tissue aging, and cellular senescence are controlled. Here we will review miRNA regulation in aging with a focus on innate immune recognition,

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human disease, and areas of active investigation that may increase our understanding of immunosenescence.

Effects of Aging on Pattern Recognition receptors (PRRs)

Key to the initiation of innate immune responses are the pattern recognition receptors (PRRs) that recognize highly conserved molecular patterns on microbes; these receptors have been shown to be dysregulated in aging [3]. The evolutionarily conserved PRRs can be divided into three branches, the Toll-like receptors (TLRs), which recognize ligands such as lipopolysaccharide, lipopeptides, flagellin, and nucleic acids [6]; and more recently characterized cytosolic NOD-like receptors (NLRs; [7]) that respond to a diverse range of signals; and RIG-I like receptors (RLRs), which are intracellular sensors of viral RNA [8]. Studies in human and animal models across cell type, activation state, and tissue context demonstrate altered expression and functional efficiency of TLRs and downstream signalling events that lead to dysregulation of immune responses in aging [3]. In human neutrophils and monocytes, decreased surface expression of TLR1 has been associated with diminished TLR1/TLR2-induced cytokine production and vaccine responsiveness [9–11]. TLR-dependent expression of the costimulatory molecules CD80 and CD86 *in vitro* was altered in monocytes from older adults [12]. Strikingly, human monocytes show an age-associated increase in TLR5, which may be relevant to higher baseline levels of inflammation, and may be harnessed to improve vaccination of older subjects through use of flagellin as an adjuvant [13]. Human dendritic cells (DCs) show age-associated decreases in TLR1, TLR3 and TLR8 protein expression by mDCs and in TLR7 and TLR9 expression by pDCs [14, 15]. Further, decreased TLR3-mediated responses to viral infection of macrophages and DCs of older individuals has been reported as well [16, 17]. Studies of effects of aging on NLRs and RLRs in humans remain undefined but recent studies in the murine model show lower induction of NLRP3-mediated cytokines following influenza infection [18] and a role for NLRP3 in mediating inflammation and cognitive deficiencies in aging [19].

Broad role of miRNAs in immunity

Thus far more than 1000 human miRNA sequences have been identified and their functions and interactions are complex and incompletely understood [5, 20]. Recent evidence points to a fundamental role for miRNAs in regulating the quantitative and dynamic aspects of the immune responses influencing the outcomes of normal and pathogenic immune responses (Table 1) [20, 21]. Particularly well studied are the miR-29 family members, which have been identified as critical suppressors of key immunological pathways. MiR-29 is expressed in both T and B lymphocytes, in the accessory cell types of thymic epithelium, and in DCs [22, 23]. In DCs, miR-29 is upregulated in response to NOD2 signals, and modulates expression of multiple immune mediators [24]; in macrophages it modulates functional polarization inducing IL-6, TNF α and CXCL9 expression [25]. Moreover, TLR-3 activation induces up-regulation of miR-29b, -29c, -148b, and -152, miRNAs that in turn target DNA methyltransferases [26].

In innate immune cells, miR-146 represses endothelial cell and macrophage activation by inhibiting NF- κ B proinflammatory pathways [25, 27]. Some members of the miR-148 family, such as miR-148a, miR-148b, and miR-152, are negative regulators of the immune innate response and antigen-presenting capacity of DCs [28]. MiR-223 is involved in modulating the NF- κ B and NLRP3 inflammasomes and in regulating leukocyte chemotaxis via chemoattractants, processes relevant for inflammation, infection, and cancer development [29, 30]. In some cases multiple miRs have been reported to share similar regulatory activity. For example, miRs such as miR-27a, miR-29b, miR-125a, miR-146a, miR-155, and miR-222 can contribute to changes in macrophage gene expression, e.g., IL-6, TNF α , and CXCL9, that occur in different exogenous activating conditions [25].

In lymphocytes, MiR-126 is expressed in mouse and human T regulatory cells (Treg) [31]. MiR-146a is induced in human primary T lymphocytes by stimulation of the T-cell receptor, and is involved in modulating T-lymphocyte-mediated immune response [32]. MiR-155 plays a crucial role in regulating B and T lymphocyte subsets and is also involved in controlling pathogen binding of DCs [33, 34]. MiR-155 seems to exert a direct influence on the expression levels of other miRNAs in DCs and macrophages, probably modulating transcription factors [35]. MiR-221 is strongly upregulated in CD4⁺ T cells, where it increases proliferation by removing the suppression of target genes linked to proliferation and survival [36].

Critical elements of investigation

Despite excellent stability of miRNAs in fresh/frozen tissue and fluids and good preservation in formalin-fixed, paraffin-embedded tissue, miRNA detection may be hampered by several intrinsic characteristics including size, low abundance, and cell or tissue-specific or functional-state-specific expression [37]. Quantitative real-time reverse-transcription PCR is the gold standard for gene expression quantitation and among the commonest methods used to detect low miRNA levels with high sensitivity and specificity. In addition, new methods have been introduced which reduce the need for pre-amplification [38]. As quantitation of circulating miRNAs is not fully standardized, it is essential to validate findings through comparison with normal reference ranges and normalization with stable reference markers [38, 39].

Age-dependent changes in miRNAs

In aging, miRNAs both target and are targeted by a number of factors important for senescence through positive and negative feedback loops. The miRNAs that have been reported to be modulated during aging have been quantified from different tissues and organisms (Table 2). Among the miRNAs differentially expressed during aging, a shared subset seems to be broadly involved in regulation of multiple species (in bold in Table 2). Our knowledge of miRNAs is increasing rapidly, and it is already apparent that nearly all the miRNAs known to be involved in immune regulation (Table 1) show modulation during aging (Table 2), suggesting that miRNA-mediated suppression of immunological pathways plays a role in aging.

Interestingly, miR-29 is strongly upregulated in aging --- both in normal mice and in mice with progeroid syndrome [40, 41]. The expression of the miR-181 family has been proposed as a marker for immune functions [42], and the age-related decline impairs T-cell receptor sensitivity [43]. Interestingly, miR-181a has recently been identified as a miR which modulates mitochondrial functions [44], which may be relevant to the increased oxidative stress and mitochondrial dysfunction associated with aging [45]. miR-221 is deregulated in aging both in humans [46] and in mouse brain and skeletal muscle [47, 48], and miR-223 it has been linked to aging of bone marrow-derived DCs [49].

Potential mechanisms of aging-related immune changes: miRNAs in inflammaging

Mechanisms of miR action in aging have been defined in some cases, such as the effect of miR-146a, an NF- κ B-dependent target that negatively regulates cytokine production and is increased in aging [50, 51]. Similarly, miR-21 targets PTEN, an inhibitor of AKT, which leads to activation of NF- κ B [51]. Several aging-associated miRNAs target central nodes of aging-associated networks, such as insulin signalling [47], TGF- β and NF- κ B signalling [51], and the p53 pathway [52]. Notably, NF- κ B is the downstream effector activated by TLR signal transduction and leads to induction of proinflammatory and antiviral response genes.

Investigation of the expression and functions of TLR, NLR, and RLR family members in innate immune cells in aging has provided evidence for impaired downstream signalling, mainly NF- κ B mediated, and has suggested several pathways of action for miRNAs to modulate immune cell function (Figure 1). Specifically, miRs may directly target components of the TLR, NLR, and RLR signalling system, such as miR-26a targeting TLR3 [30, 53] or regulation of MyD88-dependent TLR signalling by miR-145, miR-149 and miR-203 [54]. A number of miRNAs target the NF- κ B signalling pathway including miR-329 targeting the NF- κ B p65 subunit [55] and miR-15a, -92, -155, and -223 regulating IKK subunits. In addition, miR expression may be directly regulated by TLR pathway activation or they may directly activate RNA-sensing TLRs, NLRs, and RLRs since they function as ligands in cellular and lysosomal membranes [56]. NF- κ B and the NLRP3 inflammasome are important inflammatory mechanisms that are dampened by miR-223 [30, 53].

An additional mechanism for miRNA action may be through transfer between immune cells and non-immune cells. In some cases, miRNAs may be released inside vesicles, such as exosomes, microvesicles, or apoptotic bodies [57, 58], or bind to lipoproteins (HDL/LDL) [59], or RNA-binding proteins, such as Argonaute 2 (Ago2) and Ago1 [60, 61]. Recent findings suggest that although several cell transport modalities have the capacity for miRNA transfer, exosomes are perhaps the simplest and most robust way to achieve miRNA-based signal transduction in target cells [62]. Importantly, an increased release of exosome-like microvesicles was observed in normal human fibroblasts during replicative or premature senescence [63]. Little is known about how miRNA species are sorted into exosomes and which miRNA-binding proteins may be involved. However, examination of miRNAs from macrophage-derived microvesicles showed that miR-223 is abundantly expressed and

functionally active in target cells, including monocytes, endothelial cells, and epithelial cells [64].

Even in the absence of an immune challenge, healthy aged individuals have a significantly higher basal systemic inflammatory state characterized by increased cytokine levels including IL-6, IL-1 β , TNF- α , and IL-8 [4]. The study of whether and how miRNAs are involved in this “inflammaging” is an active research area in immunology and gerontology [21, 65]. Thus, mechanisms controlling resolution of acute inflammation are crucial especially in aging and age-related diseases, since resolution failure can induce many classic and non-classic age-related inflammatory conditions. Notably, an age-related hypomethylation upstream of the miR-29b gene was recently reported in monocytes [23] and an age-associated increase in miR-29b was reported in brains of mice [41]. In macrophages and brain microglia, miR-29b functions through inhibition of the anti-inflammatory protein TNFAIP3, leading to sustained activation of NF- κ B and induction of IL-6 and TNF- α production (Figure 1) [25, 66]. Thus this miR is expected to play a role in inflammaging.

miRNAs in resolution of inflammation

Recent data indicate that specific miRNAs are involved in a novel circuit to resolve inflammation involving resolvin D1 (RvD1). In particular, miR-21, miR-146b, miR-208a, miR-203, miR-142, miR-302d, and miR-219 were reported to be selectively regulated in self-limited murine peritonitis and in human macrophages overexpressing recombinant RvD1 receptor [67]. RvD1-miRNAs target cytokines and proteins involved in immune system function, e.g., miR-146b targeting NF- κ B signalling and reducing protein levels of proinflammatory IL-8 and RANTES, miR-219 targeting 5-lipoxygenase and reducing leukotriene production, and miR-21 increasing anti-inflammatory IL-10 in response to LPS and promoting cell survival and proliferation by acting simultaneously on both proapoptotic and antiapoptotic genes [68, 69].

The age-related up-regulation of miRs playing an anti-inflammatory role, such as miR-21, miR-146, miR-223, miR-29, is likely a compensatory mechanism enacted by senescent and/or activated immune cells to restrain the excessive pro-inflammatory activity that increases during normal aging and is accelerated in progression of aging-related diseases.

Conclusions and Future Directions

In the decade since miRNAs were first identified as regulatory elements, they have been identified in many species and tissues, and proposed to play a role in myriad biological functions [52]. The identification of miRNAs in aging is the first step towards understanding the specifics of their mechanisms in that process and potentially using this pathway for therapeutic advantage. However, the tissue specific functions of circulating miRNAs in different compartments is still being defined. Approaches using miRNAs to promote clearance of senescent cells and curb inflammaging have been proposed with a view to delaying aging and age-related diseases [70]. Identification of appropriate natural vectors for miRNA delivery in healthy organisms and pathological conditions would have major

applications, since they are likely to be useful as specific diagnostic/prognostic biomarkers of human diseases and/or for delivery of therapeutic miRNAs. Exploration of miRNA functions in immune cells through gain- and loss-of-function approaches may provide insights for how to restore immune equilibrium in age-related inflammatory diseases [21].

The recent observation that aging retardation and lifespan extension are achieved in mice by preventing aging-related brain NF- κ B activation highlights a direct link between innate immune activation and aging, even though confirmation in humans is needed [71]. NF- κ B signalling is not only a master regulator of inflammatory responses, but can also regulate several homeostatic responses such as apoptosis, autophagy, and tissue atrophy, all of which are dysregulated during aging and cellular senescence [72]. Particularly as NF- κ B is a target of multiple miRNAs, targeted use of miRNAs holds promise as a strategy to improve health span.

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Highlights

1. Short non-coding microRNAs regulate gene expression and immune responses.
2. Dysregulation of pathogen recognition receptors and responses occurs in aging.
3. MiRNAs that modulate immune functions can change during aging.
4. Aging-associated miRNAs target pro-inflammatory networks such as NF- κ B.
5. Aging-associated miRNAs could be therapeutic targets in immunosenescence.

Figure 1. Some of the most relevant miRs involved in restraining the activation of Pathogen Recognition Receptor (TLRs, NLRs, RLRs) and pro-inflammatory signalling pathways
Schematic representation of recognition receptors (TLRs, NLRs, RLRs) and signalling intermediates that are regulated by miRNAs.

Table 1

Immune-response associated miRNAs in humans

MiRNA	Cell/Tissue	Reference
29b	Dendritic cells, T and B lymphocyte, Monocytes/macrophages	[22, 23]
126	T regulatory (Treg)	[31]
146a/b	T lymphocytes, Monocytes/macrophages	[25, 32]
148a/b	Dendritic cells	[28]
152	Dendritic cells	[28]
155	Dendritic cells, T and B lymphocyte, Monocytes/macrophages	[33, 34, 73]
181	T lymphocytes	[43]
221	T CD4+ lymphocytes	[36]
223	Myeloid cells, neutrophils	[29]

Table 2
miRNAs associated with aging/senescence in different tissues and organisms

miRNAs in bold have been reported in multiple studies

MiRNA	Species	Tissue	Reference
1	Human, mouse, progeroid syndrome	Skeletal muscle, liver	[40, 74]
7	mouse	Skeletal muscle	[48]
9	human	B lymphocytes	[75]
10	mouse	brain	[47]
15a	Rhesus monkey	Skeletal muscle	[76]
17	mouse	brain	[47]
18a	Rhesus monkey	Skeletal muscle	[76]
19b	human	PBMC, plasma	[27, 77]
20a/20b	human	blood, B lymphocytes	[75, 78]
21/21*	human, mouse	plasma, PBMC, B lymph., heart	[27, 75, 77, 79]
22	mouse	brain	[80]
24	human	PBMC	[46]
26b	mouse	brain	[47]
27a	mouse	liver	[81]
29a, b,c	mouse, progeroid syndrome	Skeletal muscle, liver, brain	[40, 41]
34/34a	mouse, rat, drosphila	plasma, PBMCs, brain, liver	[47, 80, 82, 83]
92b	mouse	brain	[47]
93	mouse, rat	liver	[80, 82]
96	mouse	brain	[47]
99b	human	B lymphocytes	[75]
101a/101b	mouse	brain	[43, 47]
103	human	PBMC	[46]
106a	human	blood	[78]
107	human	PBMC	[46]
122	human	B lymphocytes	[75]
124/124a	mouse, various	brain, skeletal muscle, various	[47, 48, 84]
126	human	blood, plasma	[65, 78]
127	mouse	brain	[47]
128	human	PBMC	[46]
129	mouse	brain	[47]
130a	human	PBMC	[46, 77]
133a/b	human	Skeletal muscle	[74]
146a/146b	human, mice	Bone marrow deriv. DCs, serum	[49, 50, 85]
148a	human, mice	B lymphocytes, serum	[75, 85]
132	human	Bone marrow derived DCs	[49]
134	mouse	brain	[47]
139	mouse	brain	[47]
142	human	Bone marrow derived DCs	[49]

MiRNA	Species	Tissue	Reference
143	mouse	brain	[47]
144	human, macaques, chimp., rh monkey	brain, skeletal muscle	[76, 86]
145	mouse	brain	[47]
151	human, mouse	B lymphocytes. Brain	[47, 75]
151a-5p	human, rhesus monkey	serum	[46]
155	human, mouse	PBMC, DCs, blood, serum	[46, 49, 78, 85]
181a/181b/181c	human, mouse, rhesus monkey	B lymphocytes, serum, brain, skeletal muscle	[43, 46–48, 75, 76]
184	mouse	brain	[47]
186	mouse	brain	[47]
187	mouse	brain	[47]
192	human	B lymphocytes	[75]
200a	mouse	brain	[47]
204	mouse	brain	[47]
214	mouse	liver	[82]
221	human, mouse	PBMC, brain, skeletal muscle	[46–48]
223*	human	B lymph., bone marrow deriv DCs	[49, 75]
320	mouse	brain	[47]
326	mouse	brain	[47]
345	human	B lymphocytes	[75]
361-5p	human	B lymphocytes	[75]
382	mouse	brain, skeletal muscle	[47, 48]
383	mouse	brain	[47]
409	mouse	brain	[47]
412	mouse	brain	[47]
423	mouse	brain	[47]
426	mouse	brain	[47]
433	mouse	brain	[47]
434	mouse	brain	[47]
434	mouse	Skeletal muscle	[48]
451	Rhesus monkey	Skeletal muscle	[76]
454	human	B lymphocytes	[75]
455	mouse	Skeletal muscle	[48]
467	mouse	brain	[47]
468	mouse	Skeletal muscle	[48]
484	mouse	brain	[47]
485	mouse	brain	[47]
494	human	PBMC	[77]
496	human, mouse	PBMC, brain	[46, 47]
502	human	B lymphocytes	[75]
542	mouse	Skeletal muscle	[48]
598	mouse	brain	[47]
668	mouse	brain	[47]

MiRNA	Species	Tissue	Reference
669c	mouse	liver	[82]
698	mouse	Skeletal muscle	[48]
709	mouse	liver	[82]
720	mouse	brain	[80]
721	mouse	brain	[80]
1248	human, rhesus monkey	serum	[46]
1538	human	PBMC	[46]
1974	human	PBMC	[77]
1979	human	PBMC	[77]
let-7f	mouse	brain, heart	[47, 87]