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Extracellular microRNAs initiate immunostimulation via activating toll-like receptor signaling pathways

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Abstract

Since the discovery of the stability of extracellular microRNAs (miRNAs) in plasma and other body fluids about a decade ago, an increasing body of literature has addressed the function of extracellular miRNAs as novel regulators of gene expression. Although many of these studies have demonstrated that extracellular miRNAs modulate the target genes of recipient cells in a conventional base-pairing manner after exosome-mediated secretion and uptake of miRNAs, recent studies have shown that extracellular miRNAs can also play an unconventional role by rapidly modulating innate immunity and neuron excitation via directly binding to Toll-like receptors (TLRs). In this review, we will summarize the literature incremented from studying the direct activation of TLR signaling pathways by miRNAs and miRNA-like fragments in modulating immune responses.

Keywords: Extracellular microRNAs, Immunostimulation, Toll-like receptor

Introduction

The conventional working model for miRNAs, a class of naturally occurring small noncoding RNAs of19–24 nt in length, is to function via base-pairing with complementary sites on target mRNAs, causing either translational repression or direct mRNA degradation [1, 2]. For extracellular miRNAs, it is also generally accepted that they are taken up by recipient cells wherein they behave as endogenous miRNAs to modulate target gene expression through the base-pairing mechanism [1–6]. However, recent studies suggest that extracellular miRNAs may work in a more sophisticated manner [7–9]. For example, extracellular miRNAs, regardless of outside or inside the recipient cells, can serve as physiological ligands for Toll-like receptor 7 (TLR7, mouse) or 8 (TLR8, human), initiating dendritic cell immune responses [7] and spread of central nerve system (CNS) damage [8]. Given that this function of extracellular miRNAs is independent of their conventional role in post-transcriptional gene regulation, it reveals an intriguing and unusual working model of extracellular miRNAs. Herein, we describe the latest insights in the binding and activation of intracellular TLRs by extracellular miRNAs.

Recognition of specific miRNAs by intracellular TLR7 and TLR8

Innate immune cells play a critical role in host defense against invading pathogens, including microbial components and mitochondrial DNA fragments derived from apoptotic cells [10, 11]. To fulfill the function of detecting broad pathogen-associated patterns and danger-associated patterns, these innate immune cells express pattern recognition receptors (PRRs). TLRs are one of the most studied families of PRRs and their activation promotes both innate inflammatory responses and the induction of adaptive immunity [12]. Due to their broad and complicated function in innate immunity, TLRs may be considered a 'Swiss Army knife' of the immune system –replete with multifaceted responses for various infectious and disease states [12]. There are multiple TLRs expressed in immune cells, and of these, TLR1, TLR2, TLR4, TLR5 and TLR6 are expressed primarily on the cell surface where they recognize lipopolysaccharides or other unique molecules derived from microbes [10, 11]. As opposed to these cell surface TLRs, innate immune cells also express intracellular TLRs (i.e. TLR3, TLR7, TLR8 and TLR9)which are

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mainly localized at the membranes of endolysosomal compartments and can trigger the induction of cytokines essential for innate immune responses [10, 11, 13]. For example, Ramirez-Ortiz et al. have demonstrated that TLR7 and the adaptor molecule Myd88 can be recruited to the endolysosomal compartment by the receptor TREML4, which subsequently amplifies TLR7-induced type I interferon responses [13]. Both cell surface and intracellular TLRsare intrinsically capable of detecting foreign nucleic acids, including double-stranded RNA (dsRNA) and single-stranded RNA (ssRNA) of RNA viruses and DNA from bacteria and DNA viruses [10, 11, 14, 15]. Furthermore, a previous study by Heilet al. demonstrated that murine TLR7 or human TLR8 can recognize GU-rich ssRNA derived from human immunodeficiency virus-1 (HIV-1) [16]. Kariko et al. [14] found that small interfering RNAs (siRNAs) mediate sequence-independent gene suppression and induce immune activation by signaling through TLR3. In line with this, Kleinman et al. [15] showed that generic siRNAs may suppress choroidal neovascularization (CNV) via interacting with TLR3, thereby inducing interferon-gamma and interleukin-12 production.Therefore, serving as important RNA sensors, intracellular TLRs especially TLR3, TLR7 and TLR8 can detect unique foreign nucleic acids and thus initiate TLR-mediated innate immune responses.

Given that almost all cell types can secrete and deliver extracellular miRNAs to recipient cells via exosomes [4, 5], it is possible that secreted miRNAs can reach intracellular TLRs in the recipient cells and activate TLR-mediated signaling pathways. Several recent studies have shown that certain miRNAs can bind to immune cell *TLR7* in mice or TLR8 in humans and induce inflammatory cytokine secretion (Table 1) [7, 8, 17–25]. The study by Fabbri and colleagues [17] revealed that tumor-secreted miR-21 and miR-29a were delivered via exosomes into surrounding macrophages where they activated TLR8 (homologous to murine TLR7) to trigger a pro-inflammatory response. This finding suggests that activating the macrophage inflammatory responses by these oncomiRs may facilitate tumor metastasis. In line with this, He et al. showed that tumor-secreted miR-21 induced myoblast apoptosis in cancer cachexia via TLR7-c-Jun N-terminal kinase-dependent pathway [18]. Their study also confirmed that the pro-apoptotic activity of miR-21 is mediated through the binding to and subsequent signaling byTLR7, resulting in apoptosis of murine myoblasts. Lehmann et al. [8] reported that let-7 could serve as a signaling molecule to directly activate neuronal RNA-sensing TLR7 and cause neurodegeneration. Supporting this anomalous role of let-7 in activating the TLR7 signaling pathway, they found that TLR7-deficient mice were resistant to such neurodegenerative effect, while this susceptibility to let-7 could be restored in neurons transfected with TLR7. In agreement with this, Park et al. [19] found that let-7b induces TLR7/TRPA1-dependent single-channel activities in neurons and HEK293 cells overexpressing TLR7/TRPA1, and that intraplantar injection of let-7b elicits rapid spontaneous pain via activating TLR7 and TRPA1. Furthermore, their study also showed that the binding of let-7 with TLR7 requires the GUUGUGU motif, a core GU-rich motif that is also present in HIV ssRNA40, a known TLR7 ligand [16]. In fact, a GU-rich motif was identified in all TLR7/8-binding miRNA (GUUG for miR-21, GGUU for miR-29a and GUUGUGU for let-7b). The recognition of U and UG-rich motifs on miRNA is consistent with the involvement of TLR7 and TLR8 [26, 27], whose activation is sequence-specific [28, 29].

Table 1 Extracellular miRNAs serve as the ligand for TLR7/8

miRNA	Source	Receipt cells	Cytokine	Ref
miR-34a, −122, −133a, − 142, −146a, and -208a	plasma	macrophages and cardiomyocytes	MIP-2, TNF-α,IL-6	Chao W et al [20]
miR-29a	serum	Dendritic Cells	TNF-α, IL-6	Garzon R et al [7]
miR-let-7b	Rheumatoid Arthritis Synovial Fluid	macrophage	TNF-α, IL-8, IL-7	Shahrara S et al [21]
miR-21	extracellular vesicles from the brains of rhesus macaques	macrophage/microglial cells	IL-6, TNF-α	Fox HS et al [22]
miR-let-7c and − 21	exosomes of the neuronal cultures and developing brains	neurons		Hsueh YP et al [23]
miR-29b	exosomes from beta-cells	spleen cells	TNF-a	Bach JM et al [24]
miRNA-let-7b	dorsal root ganglion neurons	dorsal root ganglion neurons		Ji RR et al [19]
miR-21	lung cancer- and pancreatic cancer-derived microvesicles	myoblasts		Croce CM et al [18]
MicroRNA-146a	plasmacytoid dendritic cells	plasmacytoid dendritic cells		Blom B et al [25]
miR-21, − 147, and -29a	exosomes of cancer cells	macrophages	TNF-α, IL-6	Croce CM et al [17]
miR-let-7	Cerebrospinal fluid	macrophage/microglial cells	TNF-α	Lehnardt S et al [8]

By specifically delivering let-7b into tumor-associated macrophages (TAMs), Huang et al. [30] also showed that let-7b efficiently reprogrammed the functions of TAMs, reversing the suppressive tumor microenvironment and inhibiting tumor growth. Given that this GU-rich motif is shared by various miRNAs, it is highly possible that intracellular TLR7 or TLR8 can be targeted by these miRNAs under different physiological and pathophysiological settings. Earlier work by Judge et al. [31] showed that GU-rich motifs contributed to immune stimulation through interacting with intracellular TLR. In a similar fashion, miR-122, a predominant miRNA found in the liver, also contains two occurrences of an alternating U/G motif, and is associated with immune stimulation. Furthermore, modifying the U/G motif significantly reduces the immune stimulatory effect of endogenous miR-122 or miR-122-mimetic RNA [32].

Direct binding to immune cell TLRs by non-coding small RNAs from other species

Following the discovery of cross-kingdom regulation mediated by plant miRNAs in mammalian tissues and cells [3, 33], an increasing body of literature has shown that exogenous miRNAs derived from various species, including plants and viruses, play a critical role in modulating mammalian cell function [34–36]. Although these exogenous miRNAs execute their biological function in the recipient cells mainly through base-pairing the transcript of target genes in a manner of endogenous miRNA [34, 35], certain exogenous miRNAs that contain the TLR-binding GU-rich motif can also directly bind to intracellular TLRs or cell surfaces, leading to activation of TLR-mediated immune responses. In fact, given that the levels of exogenous miRNAs inside human and animals are extremely low, these exogenous miRNAs may have difficulty to be recruited by RNA-induced silencing complex (RISC)-loading complex in recipient cells. However, through the unorthodox way of directly binding to TLRs, exogenous miRNAs can elicit rapid biological responses in human and murine cells at extremely low concentration. Indeed, Cavalieri et al. [37] found that plant miRNAs bound to TLR3 in dendritic cells, thereby impairing TRIF signaling, limited T cell proliferation and dampened dendritic cell immune responses. Interestingly, their studies further showed that the anti-inflammatory efficacy was associated with various miRNAs derived from different plants, and that the immuno-modulatory effect of plant miRNA was independent of sequence or plant type. A

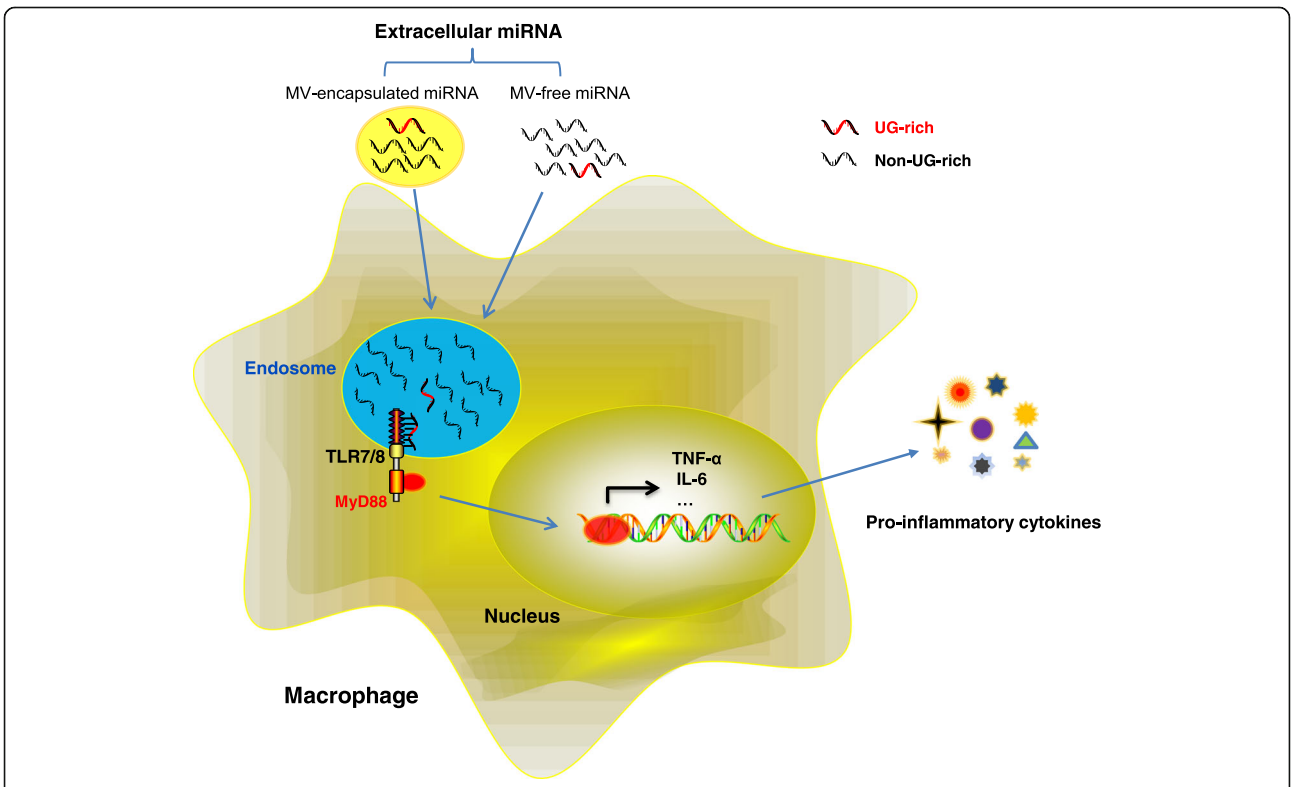

Fig. 1 Extracellular miRNAs or mIRNAs activate macrophage intracellular TLR7/8 signaling and elicit inflammatory responses. Extracellular miRNA or mIRNAs in microvesicles (MV) or MV-free condition are first internalized into the recipient macrophages, and then subsequently transported to endosomes, in which the miRNAs or mIRNAs that contain GU-rich sequence, such as miR-21 and miR-29a, bind to endosomally localized TLR7/8. After the ligation of TLR7/8 by miRNA or mIRNAs, the adaptor molecule MyD88 is recruited and drives the production of proinflammatory cytokines such as TNF-α and IL-6

previous study by Sampey and colleagues also suggested that exosomes containing trans-activating response (TAR) element RNA enhanced susceptibility of undifferentiated naive cells to HIV-1 infection [38]. TAR was found to be able to bind to TLR3, while the 5′ and 3′ stems (TAR miRNAs) bound best to TLR7 and 8, respectively. Through binding to TLRs, TAR miRNA can activate the NF-κB pathway and regulate cytokine expression and secretion. This finding provides a novel mechanism underlying the inflammation observed in HIV-1-infected patients undergoing combination antiretroviral therapy (cART) [38].

It has been generally accepted that bacteria alone cannot produce miRNAs; however, a recent study by Gu et al. [39] demonstrated that *Salmonella*, a leading cause of food-borne illness worldwide, can not only release viral non-coding RNA fragments into the infected host cells, but also hijack the host cell non-classical miRNA processing machinery to further process these viral non-coding RNAs into ~ 22-nt functional RNA fragments. Given that the bacterial 'miRNA-like' fragments generated in the host cells may also possess GU or UG-rich motifs, these bacterial 'miRNA-like' fragments can also elicit host cell inflammatory responses through binding to intracellular TLRs and activating TLR-mediated signal pathways.

Conclusion

In summary, extracellular miRNAs or miRNA-like small RNA fragments (mlRNAs) can regulate recipient cell function through both conventional and unconventional ways. Conventionally, miRNAs or mlRNAs repress protein expression at the post-transcriptional level through a base-pairing mechanism; whereas unconventionally, miRNAs or mlRNAs directly serve as ligands of TLRs. The working model of miRNAs or mlRNAs serving as TLR ligands is illustrated in Fig. 1. Extracellular miRNA or mlRNAs under microvesicles (MV) or MV-free conditions are first internalized into the recipient macrophages, and then subsequently transported to endosomes, in which the miRNAs or mlRNAs that contain the GU-rich sequence, such as miR-21 and miR-29a, bind to endosomal TLR7/8. After GU-enriched miRNA or mlRNAs sensed by TLR7/8, the adaptor molecule MyD88 is recruited to drive the production of proinflammatory cytokines including TNF-α, IL-6 and IFNγ. Although many fundamental issues remain to be further addressed, the discovery of miRNAs or mlRNAs directly serving as TLR7/8 ligands in immune cells significantly expands the field of miRNA research, and provides potentially novel therapeutic targets in controlling innate immune response and inflammation.

Abbreviations

cART: Combination antiretroviral therapy; CNS: Central nerve system; dsRNA: Double-stranded RNA; HIV-1: Human immunodeficiency virus-1; miRNAs: microRNAs; mlRNAs: miRNA-like small RNA fragments; MV: Microvesicles; PRRs: Pattern recognition receptors; RISC: RNA-induced silencing complex; siRNAs: Small interfering RNAs; ssRNA: Single-stranded RNA; TAR: Trans-activating response; TLRs: Toll-like receptors

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Authors' contributions

HL and KK searched the literature, and YL wrote the manuscript. All authors read and approved the final manuscript.

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Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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