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MicroRNAs: Regulators of the host antifungal immune response

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Highlights

- Patients are at a high risk of fungal infection during perioperative period.
- MicroRNAs are involved in inflammatory responses, pyroptosis and autophagy in antifungal immunity.
- MicroRNAs modulate the activation of Th cells during fungal infection, thereby regulating the adaptive immune response.
- Differentially expressed miRNAs could be used as fungal infection biomarkers.

Abstract

Fungi pose a severe threat to human health worldwide, especially to patients with weakened immune systems. Perioperative patients are at a high risk of fungal infection and death because of the immunosuppression caused by the surgery, as well as the use of surgical instruments. Perioperative patients in ICU are at greater risk of fungal infection. Fungal infections are often difficult to identify, and the treatment is particularly challenging. A well-functioning host immune and an appropriate level of inflammation is essential for controlling fungal infections. MicroRNAs (miRNAs) play critical roles in regulating host immune function, primarily through participating in the post-transcriptional regulation of target genes. After fungal infection, miRNAs are differentially expressed in various tissues, cells, and extracellular vesicles, promoting or inhibiting antifungal effects through different mechanisms to modulate the host immune response. In addition, differentially expressed miRNAs could serve as potential biological markers for the diagnosis and treatment of fungal infections. In this review, we discuss the role and significance of miRNAs in fungal infections.

Keywords: MicroRNA, fungal infection, antifungal immunity, inflammatory response, potential biomarkers

Introduction

There are 2.2 to 3.8 million species of fungi worldwide [1]. However, only a tiny fraction are typically associated with humans, acting as opportunistic pathogens [2]. These fungi generally inhabit in healthy individuals' mucosal and epidermal surfaces. The fungi are commensal organisms that do not cause disease under normal conditions. However, they have the potential to cause mucocutaneous fungal infections and invasive fungal infections (IFIs) in immunocompromised individuals.

IFIs are becoming increasingly common in critically ill patients, especially during perioperative period [3]. The length of hospital stay was longer among patients undergoing surgery, and prolonged hospital stay is a risk factor of hospital infection [4]. The use of central venous catheter and shunt and other medical equipment in patients with immunosuppression in the process of the operation will increase the risk of fungal infection [5, 6]. IFI can be fatal in patients receiving organ or stem cell transplantation [7]. For patients with abdominal surgery, intestinal fungi could enter blood, causing invasive fungal infections. Perioperative critically ill patients admitted to intensive care unit (ICU) are at a high risk of fungal infections. About 20% of infections in ICU are caused by fungi [8], and fungal infection is an important cause

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of death in ICU patients. IFIs have become increasingly prevalent, accompanied by high mortality [9-11]. The most common IFIs are invasive *Candida albicans* (*C. albicans*) and invasive aspergillosis. *C. albicans* is the third frequent cause of infections for ICU patients, contributing to more than 40% of the crude mortality [12-14]. Early diagnosis and immediate treatment are critical for a favorable IFI prognosis. However, clinical diagnosis of IFIs remains challenging. Fungal culture is the gold standard for the diagnosis of IFIs, but the long culture time, typically around 48 hours, can delay treatment [15].

Presently, the most common detection method for IFI is (1,3) β-D-glucan assay, which identifies the fungal-specific cell wall component (1,3) β-D-glucan in serum. The (1,3) β-D-glucan assay is a serological test for early dection with high predictive value [16]. However, (1,3) β-D-glucan are widely present in fungal cell walls, and the (1,3) β-D-glucan assay cannot distinguish fungal species. Additionally, (1,3) β-D-glucan assay sometimes shows false positive due to several factors, such as wound gauze and blood products [17, 18]. In recent years, molecular biological detection of fungal pathogen has made significant progress, such as T2 magnetic resonance assay and next generation sequencing. Molecular biology methods can detect fungi infections in a short time, but are not mature enough to replace routine microbiological, serological and histopathological examinations [19-21].

Treatment for IFIs is challenging because only three distinct antifungal drug classes are available—azoles, polyenes, and echinocandins. These drugs treat fungal infections through different mechanisms. Polyenes (Amphotericin B) directly bind to ergosterol, a crucial component of the fungal cell membrane [22]. Azoles (fluconazole, voriconazole, and posaconazole) inhibit ergosterol biosynthesis [23]. Echinocandins directly inhibit β-1,3-glucan synthesis, resulting in a loss of cell integrity [24]. All these drugs are toxic to the host, e.g., nephrotoxicity and hemotoxicity from polyenes, hepatotoxicity from azoles, and headaches and hepatotoxicity from echinocandins. Also, similar to the antimicrobial resistance of bacteria, the long-term use of broad-spectrum antifungal agents has also resulted in the development of resistance in numerous fungal strains, making it difficult to cure [25]. Therefore, healthy host immune system is vital for defending against fungal infections.

The host immune response and modulation of the immune response play critical roles in fungal infections. MicroRNAs (miRNAs) have been reported to be involved in regulating immune system [26, 27]. The dysregulation of miRNA expression can lead to immunological dysfunction. In addition, aberrant expression of miRNAs has been confirmed to be implicated in various types of diseases. As a result, miRNA expression profiles can serve as biomarkers for the diagnosis, prognosis, and treatment response of certain diseases [28-30]. Furthermore, miRNAs have recently been found to play crucial roles in antifungal immunity. This review summarizes recent research progresses on the role of miRNAs in antifungal immunity. We believe that understanding the mechanisms of miRNAs-mediated antifungal immunomodulation would be helpful for developing more effective methods to combat fungal infections in the perioperative period.

The role of miRNAs in antifungal immunity

miRNAs and their role in immunity

miRNAs are short non-coding RNA molecules that range in length from 18 to 23 nucleotides. They are capable of regulating gene expression post-transcriptionally by reducing or preventing the translation of target messenger RNAs [31]. Primary miRNA transcripts are transcribed to form precursor miRNA molecules. As precursor miRNAs are exported to the cytoplasm, they are further processed into miRNA-5p and miRNA-3p by the Ribonuclease III domain of Dicer. To date, miRNAs have been identified to regulate most of all protein-encoding genes and play critical roles in varying biological processes and diseases. miRNAs target multiple mRNAs, and each messenger RNAs may be regulated by several miRNAs [32-35].

miRNAs are involved in regulating both innate and adaptive immunity. miR-155-dependent downregulation of MyD88 suppresses the inflammatory response [36]. Downregulation of miR-885-5p promotes nuclear factor-κB (NF-κB) pathway activation and immune recruitment in cutaneous lupus erythematosus [37]. Members of the miR-146 family act as key molecular brakes to coordinately control germinal center reactions to generate protective humoral responses without eliciting unwanted autoimmunity [38]. Immune defense plays an important role in fungal infections, and miRNAs regulate antifungal immunity by regulating immune response (**Table 1**).

Table 1. Differentially expressed microRNAs following fungal exposure

miRNA	Regulation	Fungal species	Exposure model	Function in antifungal immunity	Reference
miR-132-5p	↑	*C. albicans* *A. fumigatus*	DC (human)	miR-132-5p may enhance adaptive immunity by increasing T cell activation through the regulation of FKBP1B.	[64]
miR-140-5p	↑	*C. albicans*	RAW264.7	miR-140-5p may target TGF-β1 and promote inflammatory response through the MAPK signaling pathway.	[44]
miR-146a	↑	*C. neoformans*	THP-1	miR-146a negatively regulates NF-κB activation by targeting IRAK1 and TRAF6 and inhibits *C. neoformans*-induced release of inflammatory cytokines in monocytes.	[57]
	↑	*C. albicans*	THP-1	miR-146a inhibits the activation of p-IκBα and the translocation of NF-κB p65.	[55]
	↑	*A. fumigatus*	THP-1	miR-146a negatively regulates TNF-α and IL-6 secretion.	[56]
miR-124	↓	*C. albicans*	mice	miR-124 targets MCP-1 to reduce the excessive inflammatory response.	[60]
miR344b13p	↓	*A. fumigatus*	NR8383	miR-344b-1-3p inhibits macrophage autophagy by targeting TLR2.	[54]
miR-155	↑	*C. albicans*	DC (human)	miR-155 inhibits proinflammatory cytokine secretion by targeting NF-κ B p65 and BCL-10.	[22]
	↑	*C. albicans*	MH-S	miR-155 can target SOCS1 to enhance the inflammatory response.	[49]
miR-142-3P	↓	*A. fumigatus*	$CD4^+$ T	miR-142-3p inhibited $CD4^+$ Th1 activation and targets RICTOR to reduce IFN-γ expression.	[69]
miR-384-5p	↑	*C. albicans*	RAW264.7 and J774A.1	miR-384-5p can target PGC-1β and enhance the activation of NF-κB, MAPK, and AKT inflammatory signaling pathways.	[78]

Note: miRNA, microRNA; *C. albicans*, *Candida albicans*; A. fumigatus, Aspergillus fumigatus; DC, dendric cell; FKBP1B, FK506-binding protein 1B; TGF-β1, transforming growth factor-β1; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; TNF-α, tumor necrosis factor-α; IL, interleukin; IRAK1, interleukin receptor-associated kinase 1; TRAF6, tumor necrosis factor receptor-associated factor 6; MCP-1, monocyte chemoattractant protein-1; TLR, toll-like receptor; BCL-10, B-cell leukemia/lymphoma-10; MH-S, macrophages; SOCS1, suppressor of cytokine signaling 1; CD4, cluster determinant 4; RICTOR, rapamycin-insensitive companion of mTOR; IFN, interferon; PGC-1β, peroxisome proliferator-activated receptor gamma co-activator 1β.

Inflammatory responses

Fungi, when sensed by individual and synergistic pattern recognition receptors, initiate intricate signaling cascades in innate immune cells. These cascades mediate the production of pro-inflammatory cytokines and chemokines, promote the recruitment and phagocytosis of immune cells, enhance the generation of reactive oxygen species, and improve the killing capacity of the phagocytes. Additionally, these processes influence the activation of Th cell responses [39]. An appropriate level of inflammation is essential for controlling fungal infections, but excessive inflammatory response can damage normal tissues.

miR-155

miR-155, up-regulated in both lymphoid and myeloid cells, is associated with inflammation [40, 41]. miR-155 is one of the first miRNAs discovered to participate in antifungal immunity. Heat-killed *C.albicans* could up-regulate miR-155 in bone marrow-derived macrophages. NF-κB, an important regulator of pro-inflammatory genes, is indepensible for miR-155 transcription. The C-Type lectin receptor Dectin-1, a major pattern recognition receptor in antifungal immunity, is essential for recognizing unique component of fungi cell wall-β-glucan and controlling fungal infections. NF-κB may be the primary downstream molecule of Dectin-1 that regulates pri-miR-155 transcription in macrophages [42]. Spleen tyrosine kinase (SYK) is the upstream molecule of NF-κB. Daniel et al.

showed that Dectin-1 orchestrated miR155 upregulation in a SYK-dependent manner. By activating toll-like receptor (TLR) 2 or 4, *C. albicans* reduces the accumulation of mature miR-155, though the mechanism is unclear [43]. Large amounts of chitin produced by fungi can induce the expression of TLR2 and TLR4 as well as miR-155. Chitin treatment can inhibit the expression of suppressor of cytokine signaling 1 (SOCS1) and SH2 domain-containing inositol polyphosphate 5 phosphatase 1 (SHIP1), which are known targets of miR-155 [44]. Moreover, knock-down of miR-155 can suppress inflammatory factors and cell apoptosis through upregulation of SOCS1 [45]. According to these findings, miR-155 enhances the antifungal effect by promoting proinflammatory chemokines.

The anti-inflammatory effects of miR-155 have also been reported to be a protective factor [31]. Studies on fungal infections discovered its anti-inflammatory actions. The Dectin-1-SYK/Raf-1-mitogen-activated protein kinase (MAPK) signaling pathway is necessary for miR-155 induction by *C.albicans*. The miR-155 molecule also inhibited the expression of pro-inflammatory cytokines induced by *C. albicans* by targeting NF-κB, p65 and B-cell leukemia/lymphoma-10 [46]. In addition, *C. albicans* germ tube formation was markedly decreased when miR-155 was knocked down. On the contrary, the growth of germ tubes was greatly accelerated by transfection with miR-155 mimics [45]. By suppressing different targets, miR-155 plays opposing roles in antifungal immunity. miR-155 may promote the inflammatory response to enhance antifungal immunity through targeting SOCS1. By targeting NF-κB, p65 and B-cell leukemia/lymphoma-10, miR-155 exerts anti-inflammatory actions.

miR-140-5p

In response to heat-inactivated *C.albicans* stimulation, miR-140-5p is markedly elevated in RAW264.7 cells. miR-140-5p is involved in some biochemical processes such as inflammatory response, cytokine production, and cell apoptosis [47, 48]. Transforming growth factor-β1 may be a potential therapeutic target for treating *C. albicans* infection via miR-140-5p, which promotes inflammatory responses. Moreover, the MAPK signaling pathway might play a role in the regulation of TGF-β1 by miR-140-5p. Through RPS6KA1, vascular endothelial growth factor-A, and PRKAA1, miR-140-5p might participate in the mTOR signaling pathway, and through B-cell lymphoma-2, it might participate in the JAK-STAT signaling pathway [49]. The miR-140-5p can enhance the antifungal effects by activating inflammatory pathways.

miR-146a

As a member of the miR-146 family (miR-146a and miR-146b), miR-146a plays an important role in immune and inflammatory responses by negatively regulating the NF-κB signaling pathway in macrophages [50, 51]. The expression level miR-146a is increased in THP-1 cells treated with β-glucan. The interaction between Dectin-1 and β-glucan results in a long-lasting increase in miR-146a expression, which is dependent on Dectin-1-SYK-NF-κB and p38 MAPK pathways. In contrast, the levels of pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor-α (TNF-α) exhibit a rapid and transient increase. Overexpression of miR-146a can significantly suppress the production of IL-6 and TNF-α. miR-146a mimics inhibit the β-glucan-induced activity of p-IκBα and translocation of NF-κB p65. miR-146a also inhibits NF-κB promoter-binding activity [52]. Similar phenomena are observed when THP-1 macrophage-like cells are infected with *Aspergillus fumigatus* (*A. fumigatus*). The interaction between THP-1 macrophage-like cells and *A. fumigatus* results in a long-lasting increase in miR-146a expression, which is dependent on the p38 MAPK and NF-κB signaling. In *A. fumigatus*-challenged THP-1 macrophage-like cells, miR-146a overexpression can decrease TNF-α and IL-6 production. Conversely, miR-146a downregulation significantly enhances TNF-α and IL-6 levels. A study demonstrated that the crosstalk between miR-146a and the inflammation regulating p38 MAPK and NF-κB pathways might act as a fine-tuning mechanism in the modulation of the inflammatory response in macrophages infected with *A. fumigatus* [53]. The role of miR-146 in negatively regulating the NF-κB signaling pathway was also found in *Cryptococcus neoformans* (*C. neoformans*) infection. *C. neoformans* can induce elevated miR-146a expression by interacting with TLR receptors and negatively regulate NF-κB activation by targeting IL-1 receptor-associated kinase 1 (IRAK1) and TNF receptor-associated factor 6 (TRAF6), leading to the inhibition of *C. neoformans*-induced NF-κB activation and subsequent release of inflammatory cytokines from monocytes [54]. Immune cells play an anti-inflammatory role by promoting the expression of miR-146a after recognizing the different components of fungal cells. miR-146a negatively regulates antifungal immunity by inhibiting inflammatory pathways.

miR-124

miR-124 has been shown to play an important role in regulating the inflammatory response of the immune system [55]. By inhibiting pro-inflammatory cytokines, miR-124 mediates anti-inflammatory effects [56]. Fungal infections are the most common cause of sepsis, and acute kidney injury (AKI) is a frequent consequence. The activation of the pro-inflammatory response contributes to candidiasis-induced AKI. miR-124 mimics can improve the survival outcome in candidiasis-induced septic mice. Moreover, a significant downregulation was found in the serum levels of TNF-α, IL-1β, and IL-6 in septic mice treated with miR-124 mimics. Monocyte chemoattractant protein-1 (MCP-1) has been revealed to contribute to the pathogenesis of sepsis. After treatment with the broad-spectrum triazole antifungal agent itraconazole, the miR-124 expression significantly increased, and MCP-1 level decreased in the kidneys of septic mice. Additionally, over-expression of miR-124 reduced the MCP-1 expression and attenuated candidiasis-induced AKI in septic mice. Transfection with miR-124 mimics was equivalent to the effect of itraconazole in reducing excessive inflammatory response and renal lesions in septic mice [57]. Circular RNA homeodomain-interacting protein kinase 3 (circHIPK3), which is derived from the second exon of the HIPK3 gene, plays a vital role in the modulation of cell cycle, inflammatory response, and oxidative damage [58]. CircHIPK3 was reported to aggravate septic acute kidney injury (SAKI) inflammatory responses via miR124-3p/KLF6. Mechanically, circHIPK3 upregulates KLF6 expression by competitively binding to miR-124-3p, thereby promoting the binding of KLF6 and NLRP3, activating NLRP3/caspase-1-mediated pyroptosis, and eventually aggravating SAKI inflammatory responses. Downregulation of miR-124-3p attenuates the protective effect of circHIPK3 silencing on SAKI [59]. In summary, miR-124-3p can inhibit excessive inflammatory response to attenuate the fungi induced organ injury.

Th cell activation

It is vital for both innate and adaptive immune cells to collaborate in detecting and eliminating fungi. $CD4^+$ T helper cells are the most important cells of the adaptive immune response. Several effector cells can arise from naive $CD4^+$ T helper cells, including Th1, Th2, and Th17. The major $CD4^+$ T cells involved in the antifungal immune response are Th1 and Th17 [60]. Th1 cells promote phagocyte maturation and killing by secreting interferon (IFN)-γ, GM-CSF, and TNF. Since Th1 cells and Th2 cells inhibit the functions of each other, maintaining Th1/Th2 balance is vital to antifungal immunity.

miR-132

miR-132 is considered a relevant regulator of the immune response directly against *A. fumigatus* as it is differentially expressed in monocytes and dendritic cells upon stimulation with *A. fumigatus*, but not with lipopolysaccharide. Furthermore, miR-132 expression is associated with the activation of TLR2 or Dectin-1 but not TLR4 [61]. As a result of *C. albicans* infection, miR-132 expression in dentric cells is also up-regulated. On both the RNA and protein levels, miR-132-5p is validated to interact with immunologically relevant target gene FK506-binding protein 1B (FKBP1B). Moreover, a study observed an up-regulated trend of the target genes after silencing miR-132, although not statistically significant. Therefore, it is suggested that miR-132 fine-tuned the FKBP1B gene during fungal infection [62]. In a case-control study, miRNA-132 expression was significantly higher in the plasma of patients with allergic bronchopulmonary aspergillosis (ABPA) compared to patients with allergic asthma and severe asthma with fungal sensitisation and controls. miRNA-132 expression was positively correlated with serum IL-5 levels in ABPA patients [63]. The proliferation of T cells in mice was associated with FKBP1B [64]; when Th2 cells were activated, they released IL-5, which caused eosinophil activation, B cell differentiation, and mast cell degranulation [65]. Overall, a fungi-induced increase in miR-132 expression may enhance adaptive immunity by increasing T cell activation through the regulation of FKBP1B.

miR-142-3p

In patients with systemic lupus erythematosus, the activation of T cells is negatively regulated by miR-142-3p [66]. Similarly, it was observed that miR-142-3p inhibited $CD4^+$ Th1 activation after *A. fumigatus* infection [67]. $CD4^+$ T cells are the primary source of IFN-γ. By increasing the level of phosphorylated AKT *in vivo*, RICTOR can promote Th1 cell activation [68]. RICTOR was identified as a target of miR-142-3p in endothelial cells [69]. miR-142-3p expression is downregulated in *A. fumigatus*-activated $CD4^+$ T cells, and the downregulation of miR-142-3p leads to an increase in IFN-γ expression by promoting RICTOR expression. Thus, miR-142-3p negatively regulates antifungal immunity.

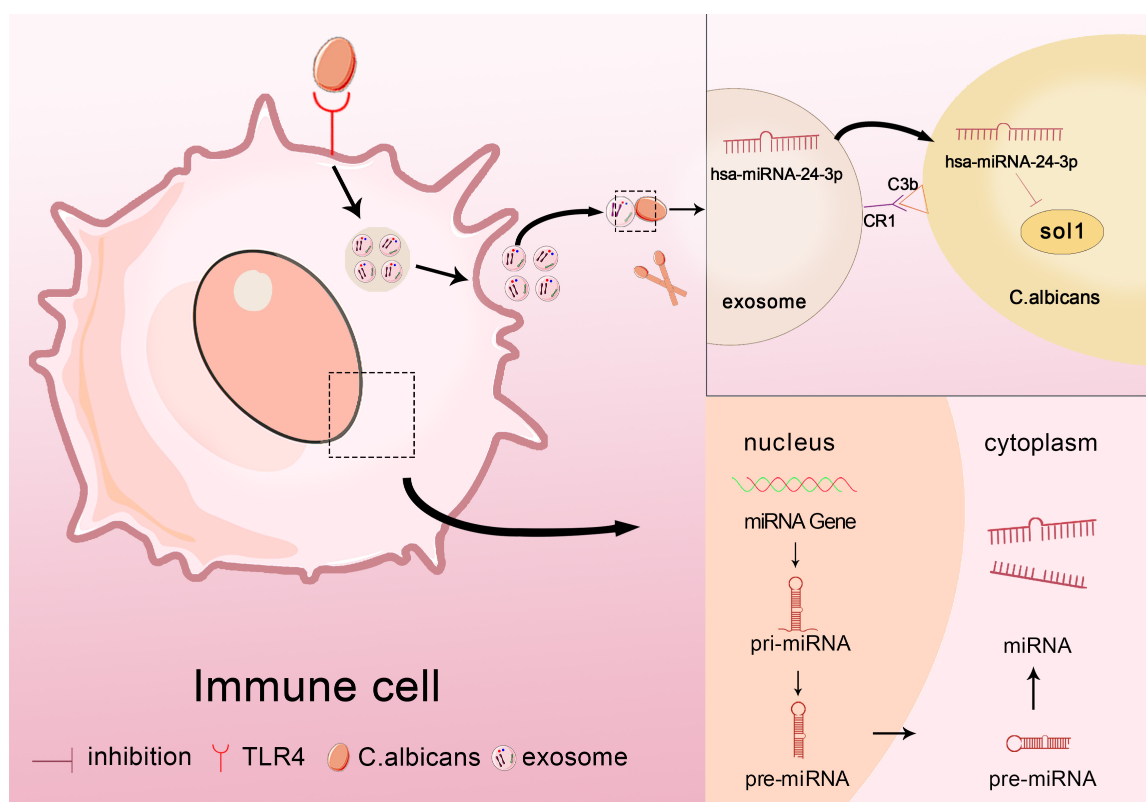

Figure 1. Interaction of *C. albicans* with miRNA-24-3p in host immune cell exosomes. Primary miRNAs are processed to form precursor miRNAs with hairpin structures in the nucleus. Precursor miRNAs are transferred to the cytoplasm and later cleaved into miRNAs of approximately 22 nucleotides in length. After *C. albicans* infection, soluble β-glucan and mannan in the cell wall of *C. albicans* bind to immune cell CR3 and TLR4 receptors, respectively, inducing the production and release of hsa-miR24-3p containing exosomes. The exosomes bind to *C. albicans* C3b via CR1, after which *C. albicans* can uptake hsa-miR-24-3p in the exosomes and inhibit the translation of sol1 mRNA, thus promoting the proliferation of *C. albicans*. *C. albicans, Candida albicans*; miRNAs, microRNAs; CR, complement receptor; TLR4, toll-like receptor; mRNA, messenger RNA.

But in a clinical study among hemato-oncology patients with profound neutropenia, free circulating miR-142-3p was significantly upregulated because of invasive aspergillosis. The reason for this difference may be related to the type of diseases [70].

Autophagy

Autophagy is an intracellular degradation mechanism that plays a crucial role in maintaining macrophage homeostasis, function, and metabolism and preventing immune senescence [71]. It is known to regulate inflammatory responses by mediating the production and secretion of cytokines, such as TNF-α and type I IFN [72]. Autophagy serves a dual role. On the one hand, it removes senescent organelles, converts them into substances such as amino acids for reuse, destroys intracellular pathogens, and promotes cell survival [73-75]. On the other hand, it induces cell death under specific caspase-independent conditions [76]. In specific tissue macrophages, autophagy enhanced NF-κB activity by sequestering A20 to boost antifungal immunity [77]. Consequently, the regulation of autophagy is a key for antifungal immunity [78].

miR-344b-1-3p

Recent studies have shown that miR-344b-1-3p negatively regulates antifungal immunity in controlling autophagy after *A. fumigatus* infection. miR-344b-1-3p can target TLR2 and negatively regulate the TLR2 signaling pathway, which is essential for activating macrophage autophagy [79, 80]. Infection of rat alveolar macrophages by *A. fumigatus* resulted in decreased miR-344b-1-3p expression. As a direct target of miR-344b-1-3p, TLR2 expression increased, thereby enhancing the activation of autophagy in macrophages. Although miR-344b-1-3p is only detected in rats at present, it is suggested that in the future, miR-344b-1-3p may be a potential therapeutic approach for aspergillosis [81].

Cross-species interaction of miRNAs

Exosomes are important signaling mediators for communication between immune cells that

Table 2. Diagnostic performance of microRNAs for invasive fungal diseases

Detection model	miRNAs	Sensitivity	Specificity	AUC	Type of disease	Reference
tetramiR	miR142-3p miR142-5p miR26b-5p miR21-5p	0.96	0.95	0.97	hemato-oncology patients with profound neutropenia	[65]
miR142-3p	miR142-3p	0.90	0.88	0.92	hemato-oncology patients with profound neutropenia	[65]
miR142-5p	miR142-5p	0.89	0.86	0.97	hemato-oncology patients with profound neutropenia	[65]
miR-26b-5p	miR-26b-5p	0.93	0.89	0.97	hemato-oncology patients with profound neutropenia	[65]
miR21-5p	miR21-5p	0.86	0.81	0.92	hemato-oncology patients with profound neutropenia	[65]
6-noncoding-RNA detection panel (4 miRNAs and 2 lncRNAs: NR_027669.1; NR_036506.1)	miR-215a mir -let- 7c miR-154 miR-193a	0.944	0.910	0.927	transplanted patients	[80]

Note: miRNA, microRNA; AUC, area under the curve.

can shuttle miRNAs to recipient cells. After infection, human immune cells release exosomes to coordinate the immune response. Exosomes are vesicles that contain various cellular components, including proteins, nucleic acids, and significant miRNAs [82, 83]. A recent study has demonstrated that miR-24-3p in exosomes released by human monocytes participates in cross-species evasion via *C. albicans* [84]. Hsa-miR-24-3p is the most common miRNA in exosomes released by human monocytes in response to the pathogenic fungus *C. albicans*. In the early stages of *C. albicans* infection, soluble β-glucan and mannans in *C. albicans* induce the production and release of hsa-miR-24-3p transport vesicles by binding to host blood monocyte complement receptor 3 and TLR4 receptors. These RNA-transport vesicles attach to the fungal surface via complement receptor 1 on the vesicle surface, after which *C. albicans* can uptake has-miR-24-3p. The host-derived hsa-miR-24-3p interacts with *C. albicans* sol1 mRNA to inhibit sol1 mRNA translation, which in turn accelerates the growth of *C. albicans* (**Figure 1**). Other miRNAs induced by *C. albicans* may also promote the survival of *C. albicans* in the host through unknown mechanisms, suggesting that miRNA inhibitors may be used to treat candidiasis.

miRNAs as potential biomarkers for fungal infection

Invasive fungal infections have high morbidity and mortality in perioperative period. In recent years, the incidence of fungal sepsis has been increasing. An accurate early diagnosis is beneficial to reducing the mortality and improving patient outcomes, but all the commonly used clinical methods for early diagnosis of fungal infections present some limitations. miRNAs with differential expression in cells, blood, and tissues have been potentially helpful for the ancillary diagnosis of fungal diseases (**Table 2**). Previous study detected differentially expressed miRNAs in peripheral blood samples from patients diagnosed with *Aspergillus* infection and found that miR-142-3p, miR-142-5p, miR-26b-5p, and miR-21-5p were significantly upregulated. After confirming a significant association between these four miRNAs and invasive aspergillosis, this group of miRNAs was subsequently labeled as a novel group of biomarkers supporting the diagnosis of invasive aspergillosis (the tetramiR group), after which the diagnostic power and accuracy of this group were evaluated. The receiver operating characteristic analysis demonstrated an area under the curve (AUC) of 0.97. Although the limited number of patients may introduce bias to determine the optimal diagnostic cutoff for this approach, there is potential value in using tetramiRs for diagnosing aspergillosis [70]. After fungal infections, miR-215 and miR-let-7c levels were up-regulated in the plasma of transplanted patients, while miR-154, miR-193a levels were down-regulated in their peripheral blood mononuclear cells (PBMCs). These four miRNAs were modeled with two lncRNAs which high expressed in PBMCs after fungal infections. The detection panel showed excellent diagnostic efficacy, with an AUC of 0.927 [85]. Another study compared the

levels of circulating miRNA between patients with paracoccidioidomycosis (PCM) and healthy controls and found that 8 miRNAs (miR-132-3p, miR-604, miR-186-5p, miR-29b-3p, miR-125b-5p, miR-376c-3p, miR-30b-5p, and miR-423-3p) were differentially expressed in the serum samples from PCM patients. The miR-132-3p expression was more than 10-fold higher in patients with PCM infection than in healthy individuals. The researchers concluded that given the relative difficulty in diagnosing PCM, these differentially expressed circulating miRNAs could be novel diagnostic biomarkers for PCM [86]. As noted earlier, miR-132 plays a role in ABPA detection and distinguishing ABPA from allergic asthma and severe asthma with fungal sensitisation. miR-132, a specifically elevated miRNA after fungal infection, can also be used as a potential diagnostic biomarker to distinguish bacterial and fungal infections.

Conclusion

This review summarizes differentially expressed miRNAs after fungal exposure and their roles on host immune responses. The expression of miRNAs varies in different cells after fungal infections, and both species and morphology of the fungi affect miRNA expression. These differentially expressed miRNAs regulate the host immune response after fungal exposure through different mechanisms. miR-155 exerts pro-inflammatory and anti-inflammatory effects by targeting different signaling pathways. miR-146 inhibits the expression of inflammatory cytokines and has an anti-inflammatory effect on antifungal immunity. miR-384-5p targets peroxisome proliferator-activated receptor gamma co-activator 1β (PGC-1β), enhancing the activation of NF-κB, MAPK, and AKT inflammatory signaling pathways, thereby exacerbating *C. albicans*-induced acute lung injury [87]. Moreover, some miRNAs prevent organ damage by suppressing excessive inflammatory responses after fungal exposure. Differentially expressed miRNAs are also involved in regulating other immune response processes. miR-344b-1-3p can regulate cellular autophagy after *A. fumigatus* exposure. *C. albicans* can induce the release of exosomes from the host and use miR-24 in exosomes to achieve accelerated growth. In addition, differentially expressed miRNAs, such as miR-132, can contribute to early diagnosis of fungal infections.

The detection technology of miRNAs has been improved. Although many articles have demonstrated miRNA expression profiles after fungal exposure, there is currently a limited number of in-depth studies on the mechanisms by which miRNAs are involved in the immune response. Understanding the regulation mechanism of miRNA in antifungal immunity can help us to deepen the understanding of fungal infections and host immunity, as well as offer potential novel therapeutic targets for fungal infections.

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