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Review

# Innate immune signal transduction pathways to fungal infection: Components and regulation

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ABSTRACT

*Candida* species are significant causes of mucosal and systemic infections in immune compromised populations, including HIV-infected individuals and cancer patients. Drug resistance and toxicity have limited the use of anti-fungal drugs. A good comprehension of the nature of the immune responses to the pathogenic fungi will aid in the developing of new approaches to the treatment of fungal diseases. In recent years, extensive research has been done to understand the host defending systems to fungal infections. In this review, we described how pattern recognition receptors senses the cognate fungal ligands and the cellular and molecular mechanisms of anti-fungal innate immune responses. Furthermore, particular focus is placed on how anti-fungal signal transduction cascades are being activated for host defense and being modulated to better treat the infections in terms of immunotherapy. Understanding the role that these pathways have in mediating host anti-fungal immunity will be crucial for future therapeutic development.

## 1. Introduction

*Candida* species are ranked as the fourth-greatest cause of hospital-acquired bloodstream infections, and are the most common human fungal pathogens with up to 40% mortality. *Candida* species grow asymptomatically in human gastrointestinal tract and skin in immunocompetent individuals. However, under certain condition, *Candida* species can cause severe mucosal and systemic infections. Risk factors include central venous catheter implants, major surgeries such as neutropenia, organ transplants, cancer therapy, and HIV infection. Current anti-fungal drugs include three major classes - polyenes, azoles, and echinocandins (Bustamante, 2005; Eggimann et al., 2003; Horn et al., 2009). However, drug resistance and toxicity (Perlin, 2015; Whaley et al., 2016) have inhibited the efficacy of anti-fungal medicines.

To protect the host from fungal pathogens including *Candida* species, the host immune system utilizes innate immune cells such as macrophages and neutrophils as the first line of defense to clear pathogens. A deep understanding of the mechanisms by which host-pathogen interacts and immune responses to fungal dissemination, will assist in developing immune-based strategies to combat fungal infections. Here we focus on the signal transduction events upon receptor clustering and engagement by fungal ligands. We also summarize recent discoveries of the mechanisms whereby the modulation of key molecules involved in the cascades facilitate host anti-fungal outcomes. These studies provide thoughtful insights for developing new therapeutic anti-fungal approaches.

## 2. Fungal recognition by PRRs

The *C. albicans* cell wall is firm while dynamic, and essential for fungal viability since it functions as a rigid but adaptable layer to sustain cell shape. It also expresses important fungal virulence proteins such as Candidalysin (Naglik et al., 2019) and is the first line of defending against host immune system. The *C. albicans* cell wall can be viewed as a layered structure, comprised of an outer layer of glycosylated proteins with 80%–90% mannose, and an inner layer of polysaccharides β (1,3)-glucan, β (1, 6)-glucan and chitin (Free, 2013). Each type of polysaccharides of the *C. albicans* cell wall is important pathogen-associated molecular patterns (PAMPs) and exhibit immunomodulatory properties. They are recognized by cognate pattern recognition receptors (PRRs) on the immune cells (Gow et al., 2007), which subsequently induce immune responses

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towards fungal pathogens.

β-1,3-glucan, which constitutes ~40% of cell wall dry weight of *C. albicans*, is detected by Dectin-1, a well-studied PRR for promotion of fungicidal activity for immune cells (Brown, 2006; Brown et al., 2002, 2003; Chen et al., 2019; Goodridge et al., 2011). Dectin-1 belongs to the C-type lectins (CLRs), and is a type II transmembrane protein highly expressed on myeloid-derived phagocytes (monocyte/macrophage, dendritic cells, and neutrophil lineages) and required for proper modulation of immune responses (Taylor et al., 2002). Dectin-1-deficient leukocytes exhibit significantly impaired responses to fungal infections even fungi was being treated with opsonins. Impaired leukocyte responses include reduced inflammatory cell recruitment and enhanced fungal dissemination (Taylor et al., 2007). Dectin-1 contains an extracellular carbohydrate recognition domain, and an intracellular immunoreceptor tyrosine-based activation (ITAM)-like motif. Dectin-1 activation trigger recruitment and activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which leads to release of antimicrobial reactive oxygen species (ROS) into the phagosome (Underhill et al., 2005). Dectin-1 activation also triggers inflammatory responses, including production of the proinflammatory cytokines. These elements recruit other immune cells to the site of infection, leading ultimately to the differentiation of $CD4^+$ T helper 1 and 17 (Th1 and Th17) for long-term memory of fungal infection (Taylor et al., 2007).

The outer layer of the glycosylated cell wall proteins is also essential for the immune recognition during fungal infection. The mannan layer has complicated biochemical polysaccharide makeup, it is conceivable that several PRRs participates in the immune recognition. For instance, mannose receptor detects α-(1, 2)→(1, 3) mannose of *N*-mannan, TLR2 (Toll-like receptor 2) targets phospholipomannan and TLR4 targets *O*-mannan, while Dectin-2, -3 and Galectin-3 participate in α-mannan and β-mannan recognition respectively (Netea et al., 2006). Wang et al. recently identified the *C. albicans* small secreted cysteine-rich protein Sel1 is a novel PAMP for TLR2 and TLR4 (Wang et al., 2019). Several TLRs including TLR2 and TLR4 coordinate with Dectin-1 for fungal recognition (Gantner et al., 2003; Viens et al., 2022; Wang et al., 2019; Willcocks et al., 2013). TLRs link the downstream adaptors MyD88 (myeloid differentiation primary response 88) to activate the signaling including nuclear factor (NF)-κB and mitogen-activated protein kinases (MAPKs) such as extracellular signal regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 for the induction of proinflammatory cytokines and chemokines (Gantner et al., 2003). Li et al. demonstrated that TLR2 is also able to form heterodimers with the C-type lectin

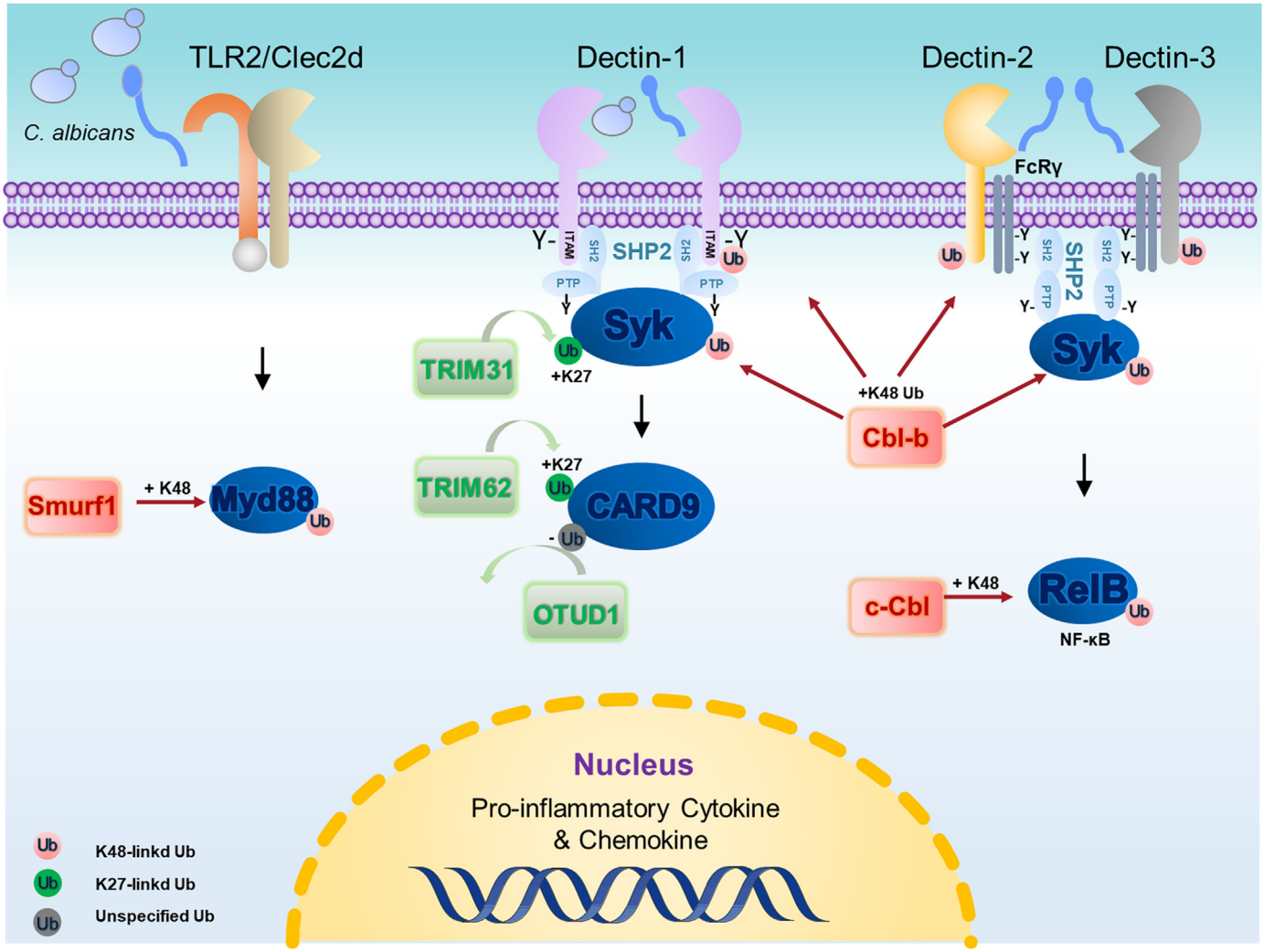


**Fig. 1. Signal transduction upon *C. albicans* infections.** Dectin-1, Dectin-2 and Dectin-3 are the main CLRs receptors for recognizing fungal ligands and summarized in this review. The tyrosine (Y) located in the YXXL motif of the intracellular domain of Dectin-1 and FcRγ adaptor chain is primarily phosphorylated by the tyrosine kinase Src. The phosphorylated YXXL motif recruit SHP2, which subsequently recruits Syk via its ITAM motif at the C-terminus. Syk then activates the CARD9 complex through PKCδ. This eventually activates the transcriptional factor NF-κB, which translocates to the nucleus to induce pro-inflammatory cytokines transcription and production. Several major CLR molecules are also being modified post-translationally by E3 ligase-mediated K48-linked, or K27-linked ubiquitination, including Cbl-b, c-Cbl, TRIM31, and TRIM62. Deubiquitination also occurs to CARD9 modulated by the deubiquitinase OTUD1.

receptor 2d CLEC2D that enhances the β-glucan recognition. The heterodimers negatively regulate anti-fungal immunity through E3 ligase Smurf1-mediated K48-linked ubiquitination and degradation of MyD88, and eventually suppressing IL-12 production (Li et al., 2023). TLR7 and TLR9, which are located on intracellular endosomal membranes, have also been implicated in antifungal immunity (Bourgeois et al., 2011; Kasperkovitz et al., 2011).

## 3. Signaling transduction upon fungal infections

Clearance of fungal pathogens including *C. albicans* starts with immune recognition of the microbes. Receptor engagements initiates phagocytosis to internalize the invading pathogens for degradation. This phagocytic event leads to locally concentrated signal cascades that are gathered with protein kinases, lipid signal transducers including phosphotidylserine (PS) and phosphoinositides (PIPs). All these events collectively promote, and coordinate phagosome maturation marked by fusion with lysosomes to form an increasingly acidified compartment for elimination of the engulfed microbes (Levin et al., 2016; Levin-Konigsberg et al., 2019). So far, tremendous work has been done to illustrate the signaling transduction cascades that are critical for antifungal immunity. We summarize the recent discoveries and the physiological significance on host immune responses to fungal infections (Fig. 1), and included the post-translational modifications (PTMs) of key molecules in CLR signaling in Table 1.

### 3.1. C-type lectin receptors

Dectin-1 mediates phagocytosis and pathogen clearance (Herre et al., 2004). The extracellular domain of Dectin-1 recognizes β (1,3)-glucan, and followed by tyrosine (Y) residue within the cytoplasmic ITAM phosphorylation by Src family kinases (Table 1). The ITAM-based signaling events lead to the spleen tyrosine kinase (Syk) recruitment (El-Hillal et al., 1997), followed by caspase-recruitment domain (CARD)-mediated aggregation of CARD9 (Gross et al., 2006) with the adaptor protein B cell lymphoma 10 (BCL-10) and the associated mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1), triggering activation of the NF-κB pathway (Bi et al., 2010), and the MAP kinases (Slack et al., 2007).

Mentrup et al. refined the degradative pathway of Dectin-1 which differs dramatically dependent on the presence of the stalk region (Dectin-1a form) or not (Dectin-1b form). The observation that Dectin-1 internalization results in the ubiquitination-mediated receptor degradation and immediate signaling shutdown, was mostly validated for the stalkless Dectin-1b form in the C57BL/6J mouse strain. Mentrup et al. reported that the Dectin-1a isoform internalization in BALB/c mice and human results in the formation of a stable receptor fragment without the extracellular ligand binding domain (Mentrup et al., 2022a). This truncated fragment persists in phagosome and continues the signal transduction through association with Syk. The eventual signaling is terminated by the intramembrane proteases Signal Peptide Peptidase-like (SPPL) 2a and 2b to cleave the residual fragment off the phagosome. Immune cells deficient in SPPL2b demonstrate increased anti-fungal ROS production, and cytokine responses (Mentrup et al., 2022b).

Dectin-2, another type of C-type lectin expressed by myeloid cells, selectively binds to hyphal mannose of *C. albicans* instead of the yeast form (Sato et al., 2006). Although the cytoplasmic region of Dectin-2 lacks the ITAM motif which is included in Dectin-1, Fc receptor γ (FcRγ) chain fills in and transduces the signal down to NF-κb for proinflammatory elements production. Saijo et al. studied the role of Dectin-2 during systemic *C. albicans* infection by using Dectin-2-deficient (Clec4n$^{-/-}$) mice. They identified that Dectin-2 is essential for *Candida* α-mannan recognition and proinflammatory cytokine expression, and Dectin-2 particularly boosts differentiation of Th17 cells that is crucial for clearing systemic candidiasis (Saijo et al., 2010). Zhu et al. further

**Table 1**
The post-translational modifications (PTMs) of key molecules in CLR signaling.

| Molecules | Regulators of PTMs | PTMs | Site | Functions |
|---|---|---|---|---|
| Dectin-1 | Cbl-b | K48-linked ubiquitination | Lys2, Lys7, Lys34 | E3 ligase Cbl-b targets Dectin-1 at Lys2/7/34 for K48-linked ubiquitination and degradation (Xiao et al., 2016). |
| | Src | Phosphorylation | Y15 | Src phosphorylates the YXXL motif of Dectin-1 to trigger signal transduction (Rogers et al., 2005). |
| Dectin-2 | Cbl-b | K48-linked ubiquitination | Lys10 | E3 ligase Cbl-b targets Dectin-2 at Lys10 for K48-linked ubiquitination and degradation (Xiao et al., 2016; Zhu et al., 2016). |
| Dectin-3 | Cbl-b | K48-linked ubiquitination | Lys 9 | E3 ligase Cbl-b targets Dectin-3 at Lys 9 for K48-linked ubiquitination and degradation (Zhu et al., 2016). |
| Src | Csk | Phosphorylation | Y530 | Csk kinase phosphorylates Src at Y530, which binds intramolecularly to the SH2 domain to inhibit Src activation (Okada & Nakagawa, 1989). |
| | Src | Auto-phosphoryaltion | Y419 | Src activation is mediated through auto-phosphorylation at Y419. |
| Syk | Src | Phosphorylation | Y525 | Src phosphorylates Syk at Y525 to fully activates Syk activity (El-Hillal et al., 1997) |
| | Cbl-b | K48-linked ubiquitination | N/A | E3 ligase Cbl-b interacts with Syk at Y317 for K48-linked ubiquitination and degradation (Wirnsberger et al., 2016; Xiao et al., 2016). |
| | TRIM31 | K27-linked ubiquitination | Lys375, Lys517 | E3 ligase TRIM31 targets Syk at Lys375 and Lys517 for K27-linked ubiquitination and promotes Syk phosphorylation (Wang et al., 2021). |
| PKCδ | N/A | Phophorylation | Y311 | Syk-mediated PKCδ phosphorylation at Y311 is essential for anti-fungal immunity (Strasser et al., 2012). |
| CARD9 | PKCδ | Phosphorylation | T231 | PKCδ-induced CARD9 phosphorylation at T231 is essential for CBM complex formation (Gross et al., 2006). |
| | TRIM62 | Ubiquitination | Lys125 | E3 ligase TRIM62 targets CARD9 at Lys125 for K27-linked ubiquitination to promote anti-fungal immunity (Cao et al., 2015). |

(*continued on next page*)

**Table 1** (*continued*)

| Molecules | Regulators of PTMs | PTMs | Site | Functions |
|---|---|---|---|---|
| | OTUD1 | De-ubiquitination | N/A | Deubiquitinase OTUD1 cleaves polyubiquitin chains off from CARD9 to negatively modulate anti-fungal signaling (Chen et al., 2021). |
| NF-κB | Raf1 | Phosphorylation | Ser276 of p65 | The kinase Raf1 phosphorylates p65 at Ser276 to positively impact T helper cell differentiation in DC cells (Gringhuis et al., 2009). |
| | c-Cbl | Ubiquitination | RelB | E3 ligase c-Cbl targets noncanonical NF-κB RelB for degradation (Duan et al., 2021). |

advanced understanding of α-mannan recognition by showing the role of Dectin-3, a previously unknown CLR in against *C. albicans* infection. Dectin-3, reported to target trehalose 6,6′-dimycolate (TDM), the mycobacteria cell wall component, forms heterodimers with Dectin-2 to sense α-mannan more effectively (Zhao et al., 2014; Zhu et al., 2013). Genetically deleted Dectin-3 in host results in highly susceptible to fungal infections by decreasing inflammatory responses (Zhu et al., 2013).

### 3.2. Src-Syk module

Src kinase is a proto-oncogene encoding a cytoplasmic protein tyrosine kinase. Src is classified as a nonreceptor tyrosine kinase belonging to the Src family kinases, which include several members with similar functions and structures. Src family kinases play critical roles in cancer progression, inflammation-related and antifungal signaling pathway (Roskoski, 2015). Src activity is regulated by the autophosphorylation of its own tyrosine residues. There are two critical tyrosine residues (Y419 and Y530 in human) and the SH2 (Src-homology 2) domain that is essential for activity maintenance. The tyrosine closest to the C-terminus (Y530) is continuously phosphorylated by Csk, a negative regulator of Src activity. The phosphorylated Y530 residue binds intramolecularly to the SH2 domain of Src, occupying the kinase site and maintaining Src catalytically inactive (Okada & Nakagawa, 1989). Upon fungal ligand binding, Src becomes activated by removing the phosphate from the C-terminal tyrosine by CD45 and CD148, two structurally related transmembrane tyrosine phosphatases (Goodridge et al., 2011). The activated Src subsequently phosphorylates the YXXL motif with the intracellular ITAM domain of Dectin-1 to trigger the signal transduction (Rogers et al., 2005).

Syk is a 72 kDa non-receptor tyrosine kinase that contains two SH2 domains and a kinase domain. Syk localizes mostly in the cytosol and keeps an autoinhibited structure in resting cells. Upon ligand ligation, Syk binds to the *p*-YXXL motif of Dectin-1 cytoplasmic tail through its SH2 domain, followed by interaction of Src with the receptor-bound Syk (Rogers et al., 2005). This dual layer of regulation results in full activation for Syk (El-Hillal et al., 1997). The dual activation mechanism of Syk has crucial physiological significance: the initial ITAM binding with Dectin-1 promotes the transition to active conformation by opening up the catalytic loop and therefore triggers rapid activation of Syk, and the subsequent phosphorylation by Src results in long-control of the active state. Syk has also been reported to trigger the autophosphorylation of its tyrosine residues at the linker region, and this aids the maintenance of Syk activity. Ten tyrosine residues in Syk that have been reported to be auto-phosphorylated, and are involved in Syk-mediated downstream signal transduction (Mocsai et al., 2010). In addition, Syk itself as a tyrosine kinase, can phosphorylate ITAMs, providing a positive feedback loop during initial binding to ITAM motif of Dectin-1.

Our laboratory identified the positive regulation of the tripartite motif 31 (TRIM31) protein in modulating Syk-mediated signaling cascades during fungal infection (Wang et al., 2021). The TRIM family has been found to be involved in multiple biological processes including combating viral infection and tumor progression by catalyzing K48- or K63-linked polyubiquitination. We discovered that TRIM31 is an essential regulator for Syk activation via catalyzing the K27-linked polyubiquitination at Lys375 and Lys517 of Syk. This promotes Syk binding to the upstream CLRs and upregulates Syk kinase activity. Consequently, TRIM31 protects hosts from systemic infection with *C. albicans* in the mouse model via increasing the production of pro-inflammatory cytokines and chemokines, which promotes the pathogen being cleared off more efficiently.

Casitas B lymphoma-b (Cbl-b), a member of the RING-finger-type E3 ubiquitin ligases, has been reported to negatively regulate a couple of signaling cascades, ranging from TCRs, BCRs, TLR4 *etc*. (Han et al., 2010; Naramura et al., 2002; Sohn et al., 2003). In the context of anti-fungal immunity, multiple groups demonstrated that Cbl-b promotes degradation of several key CLRs molecules and inhibit anti-fungal immune responses (Wirnsberger et al., 2016; Xiao et al., 2016; Zhu et al., 2016). Cbl-b targets Syk, along with three major fungal ligand receptors Dectin-1, Dectin-2, Dectin-3 for K48-linked polyubiquitination. Zhu et al. refined that the ubiquitinated CLRs are further transported to lysosomes through an endosomal sorting complex required for transport (ESCRT) system (Zhu et al., 2016). Cbl-b deficiency protects mice with enhanced anti-fungal responses, including increased inflammasome activation and elevated reactive oxygen species production. Silencing the *Cbl-b* gene expression by injection of the *Cbl-b*-specific siRNA intravenously through the tail vein or injection of a cell-permeable Cbl-b inhibitory peptide intraperitoneally protects mice from lethal systemic *C. albicans* infection. Targeting Cbl-b may hence be a promising therapeutic approach for enhancing host defense against fungal infections.

Deng et al. identified an essential role for the tyrosine phosphatase SHP-2 in regulating CLR-involved Syk activation, and it operates as a scaffold independent of its phosphatase activity in the context of fungal infections (Deng et al., 2015). SHP-2 is a cytoplasmic tyrosine phosphatase important for the signaling induced by growth factors, cytokines and hormones (Feng et al., 1993), and is composed of two tandem SH2 domains at the N-terminus, a tyrosine-phosphatase domain and a carboxy-terminal tail. They found that a previously unnoticed ITAM motif in the C-terminus of SHP-2 kinase domain is phosphorylated upon ligation of various fungal ligands. This phosphorylated ITAM motif then functions as a scaffold protein recruiting Syk to the CLR receptor, which promotes the activation of Syk-mediated signaling events. SHP-2 in myeloid cells is crucial for the induction of proinflammatory cytokines and chemokines and anti-fungal responses of the $T_H17$ subset of helper T cells in controlling *C. albicans* infection (Deng et al., 2015).

Our laboratory identified the role of STING (stimulator of interferon genes) in combating *C. albicans* infection (Chen et al., 2023) (Fig. 2). STING is essential for host immune defense against bacterial and viral infections. It senses endogenous cyclic dinucleotides, including cGAS (cyclic GMP-AMP synthase)-synthesized 2, 3-cyclic GMP-AMP (2, 3-cGAMP) (Ishikawa & Barber, 2008); it recognizes exogenous cyclic dimeric guanosine and adenosine monophosphates (c-di-GMP, c-di-AMP) released from bacteria (Zhang et al., 2020). STING activation subsequently provides protection against the invading viral and bacterial pathogens by triggering autophagy and biosynthesis of interferon (Gui et al., 2019). We found the negative impact for STING in regulating activation of CLR-associated signaling cascades towards fungal infections. Upon fungal stimulation, STING transits to the phagosomal membrane that confines the fungi. This dramatically differs from the conserved trafficking route where STING moves to the Golgi apparatus from the ER when activated by cGAMP. In phagosome, STING binds with Src via its first 18 amino acids directly, and this inhibits the formation of Syk and Src complex demonstrated by biochemical results. Src catalyzes

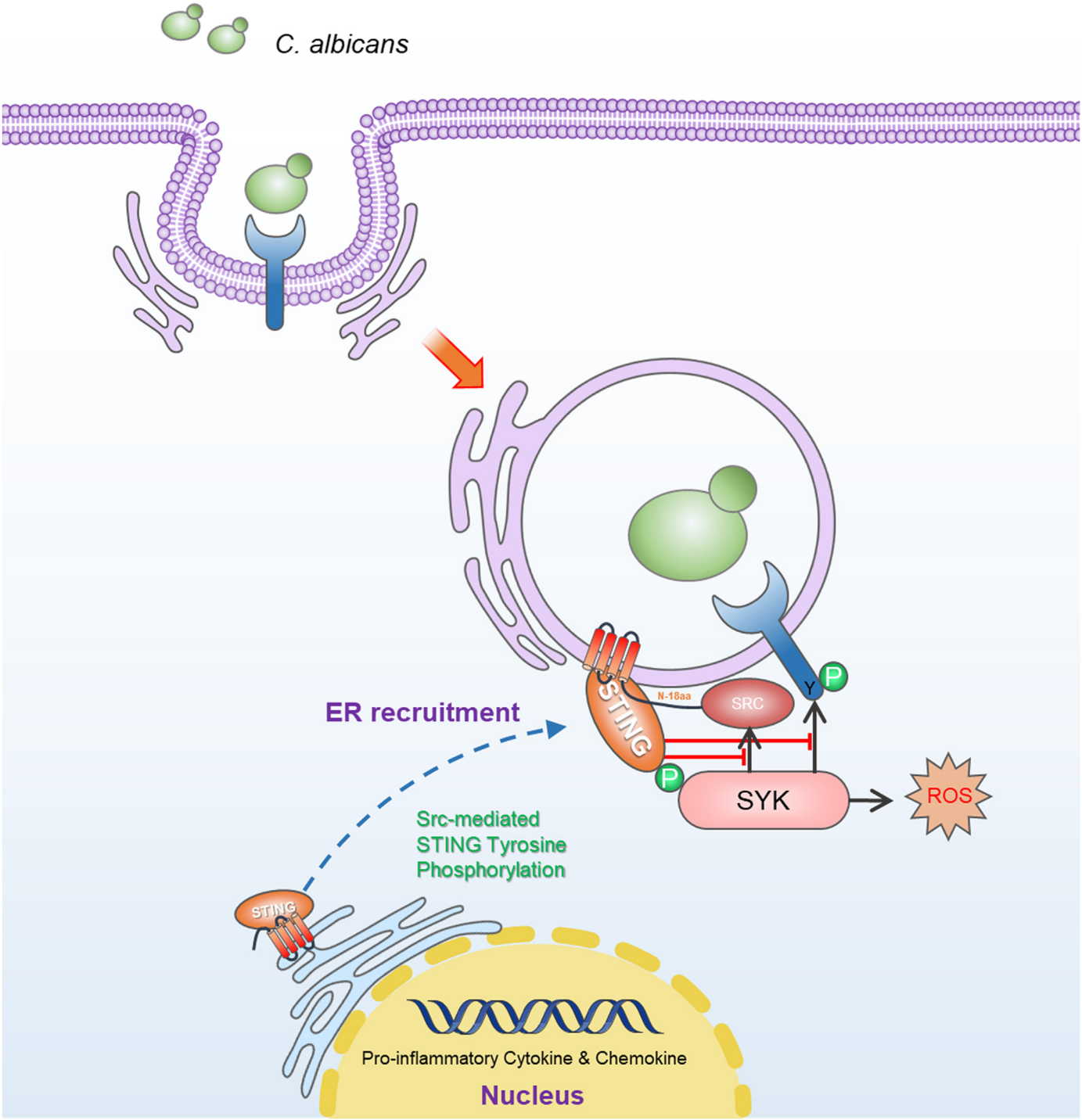


**Fig. 2. The nucleotide receptor STING negatively modulates antifungal immunity through inhibition of CLRs-associated signaling cascades.** *C. albicans* infections stimulate STING to translocate to the fungi-containing phagosome. In phagosomes, STING forms complex with Src and inhibits the recruitment and activation of Syk. Src triggers the tyrosine phosphorylation of STING at Y245 locus, and this further prevents Syk being recruited to the Dectin-1 receptor.

STING tyrosine phosphorylation at Y245 locus, which further suppresses Syk activation. It was found that STING negatively controls anti-fungal immunity *in vivo*, and administration of the first 18-aa peptide of STING improves the survival rate of mice in the systemic model of *C. albicans* infections (Chen et al., 2023).

### 3.3. *PKCδ-mediated CARD9 activation*

Protein kinase C (PKC) family play major roles in human diseases progression (Garg et al., 2014). Strasser et al. established an essential role for PKCδ which couples CLR proximal events to CARD9 activation (Strasser et al., 2012). Fungal ligand treatments induce PKCδ phosphorylation at the Y311 locus, and this induction is via the CLR signaling, including the upstream kinases Src and Syk. PKCδ further engages CARD9 and controls the activation of the canonical NF-κB activation to selectively modulate the proinflammatory cytokine production but not phagocytosis nor ROS secretion. Dendritic cells with *Pkcδ* deficiency, but not those lacking PKCα, PKCβ, PKCβ and PKCθ exhibit impaired innate

responses to CLRs stimulation. *C. albicans*-induced cytokine production is dampened in immune cells lacking PKCδ, and mice with PKCδ deleted survive better and have significantly less weight loss upon infections.

Gross et al. discovered that CARD9 is a key transducer downstream of PKCδ signaling (Gross et al., 2006). Although not involved in TLR- or BCR-induced signaling, Card9 controls innate anti-fungal immunity and Dectin-1-mediated myeloid activation (Hara et al., 2007). Activated Syk engages PKCδ to phosphorylate CARD9 at the threonine 231 locus (Strasser et al., 2012). Phosphorylated CARD9 interacts with the CARD domain of BCL10 and forms a CARD9-BCL10-MALT1 (CBM) complex, and this initiates the conserved NF-kB pathway (Hara et al., 2007). On the other hand, Jia et al. found upon that stimulation of *C. albicans* yeasts or curdlan, a type of β (1,3)-glucan polymer produced from *Alcaligenes faecalis,* CARD9 is not involved in Dectin-1-induced NF-κB activation but instead is indispensable for Dectin-1-induced ERK MAPK activation. In this scenario, Syk is activated upon β (1,3)-glucan stimulation, and phosphorylates Ras-GRF1, which activates H-Ras and recruits CARD9 to form a complex. This eventually leads to ERK activation (Jia et al., 2014). Mice lacking of CARD9 expression is more susceptible to diverse fungal infections, including *Candida albicans, Cryptococcus neoformans*, and *Aspergillus fumigatus* and intracellular pathogen *Listeria monocytogenes* (Campuzano et al., 2020; Hsu et al., 2007; Rieber et al., 2016; Zhang et al., 2021).

The N-terminus of CARD9 is composed of a CARD domain followed by two coiled-coil domains that serves as an oligomerization domain. The C-terminus of CARD9 is essential for regulating its activity. Truncation of the exon 11 of CARD9 (Δ11) at C-terminus shows significant impairment of proinflammatory cytokines expression upon fungal ligand stimulation (Cao et al., 2015). Inversely, upon fungal infections, the autophagy inhibitor protein Rubicon (Beclin-1-interacting cysteine-rich-containing) is found to switch the binding partner to interact with CARD9 and disassemble it from the CBM complex. Rubicon is hence a feedback inhibitor to terminate CARD9 signaling and coordinate the immune responses to suppress excessive proinflammatory cytokines production (Yang et al., 2012).

Ubiquitination of CARD9 is also key to control its activity. TRIM62 binds the C-terminus of CARD9 and catalyzes K27-linked polyubiquitination at the K125 locus of CARD9 (Cao et al., 2015). The K125R mutation terminates CLR-induced CARD9-mediated cytokine production upon fungal ligand stimulation. Furthermore, *Trim62*-deleted mice exhibit increased susceptibility to fungal infection. Our laboratory screened the deubiquitinases (DUBs) and found the ovarian tumor (OTU) DUB family member OTU DUB 1 (OTUD1) as a critical activator of CARD9 function(Chen et al., 2021). OTUD1 directly interacts with CARD9 and cleaves polyubiquitin chains off CARD9. OTUD1 deficiency down-regulates the CARD9-mediated signaling and dampens production of the proinflammatory elements upon fungal stimulation. *Otud1*-deficient mice are therefore more sensitive to systemic fungal infection *in vivo*. Our results indicated that OTUD1 plays a major role in CLR-associated CARD9 signaling in antifungal immunity.

### 3.4. NF-κb

The NF-κB family is composed of five members that form hetero- and homo-dimers: the transcriptionally active subunits p65, c-Rel and RelB, and p50 and p52, the latter two lack transactivation domains (Gringhuis et al., 2009). NF-κB dimers are normally inactive in the cytoplasm in resting cells and move into the nucleus after activation by various stimuli. The conserved NF-κB activation cascade starts with activation of the IκBα kinase (IKK) complex, which is controlled by phosphorylation of the catalytic subunits IKKα and IKKβ. The activated IKK complex subsequently phosphorylates IκBα proteins, followed by ubiquitination-dependent degradation of IκBα. This leads to NF-κB translocation into the nucleus for the activation of cytokine genes.

Gringhuis et al. showed that the serine/threonine kinase Raf1 is important for inducing $T_h1$- and $T_h17$- polarizing cytokines production in response to both curdlan and *C. albicans* infection (Gringhuis et al., 2009). Raf-1 has been reported to be involved in cell proliferation, oncogenic transformation, and apoptosis (Romano et al., 2014). In the context of fungal infections, Dectin-1-mediated Syk signaling activates the canonical p65 and c-Rel, and the noncanonical RelB. The kinase Raf1 is found to be activated downstream of Dectin-1 signaling cascade and modulate RelB activity in a Syk-independent manner. The mechanism that Raf1 modulates pro-inflammatory cytokines expression is achieved through two layers: 1. Raf-1 phosphorylates p65 at the Ser276 locus that promotes the formation of inactive p65-RelB dimers. Repression of RelB inversely induces the expression of IL-12p40 and IL-1β by recruiting more RNA polymerase to the promoter region of the cytokine genes; 2. p65 phosphorylation at Ser276 is prone to its acetylation by histone acetyltransferase and therefore increases its DNA affinity and transactivation activity. Raf1 is essential to T helper cell differentiation by influencing cytokine expression such as IL12p70 and IL23, and hence being crucial in the induction of adaptive immunity to *C. albicans* (Gringhuis et al., 2009).

Duan et al. identified that the c-Cbl (Casitas B-lineage lymphoma), a RING finger E3 ubiquitin ligase is involved fungal induced intestinal inflammation through ubiquitinating and degrading RelB (Duan et al., 2021). Mice with DC-specific deletion of c-Cbl are more prone to DSS (dextran sodium sulfate)-induced colitis, shown by increased bowl inflammation such as neutrophils infiltration in the colon. c-Cbl performs the inhibitory effects of bowl inflammation via degrading RelB upon α-mannan stimulation. Degradation of RelB makes it no longer repress p65-mediated transcription of IL-10, a type of protective anti-inflammatory cytokine in inflammatory bowel disease. The *c-Cbl*-deficient BMDCs treated with α-mannan therefore has lower expression of IL-10. Inhibiting fungal growth by antifungal agent fluconazole, or inhibition of RelB activation *in vivo* aids in improving colitis in mice with *c-Cbl*-deficient (Duan et al., 2021).

## 4. Connections between fungal microorganisms and cancer progression

Currently, the importance of the microbiome for human health and disease, particularly cancer pathogenesis and prognosis has become increasingly evident. Our body microbiota is composed of bacteria, fungi, and viruses (Li et al., 2019). Many studies have demonstrated the role of bacteria and viruses in tumor progression, whereas the impact of fungal microorganisms (mycobiota) remains largely unknown. Although it accounts for a low biomass compared to the bacterial load, the mycobiota has an unquestionable impact on health and disease. Not only are fungi significant commensals within the human gastrointestinal tracts but they also demonstrate as opportunistic pathogens especially in individuals with immunocompromised immune systems, such as HIV-infected population and patients with cancer.

Gut mycobiota is an essential component of the intestinal microbiome and comprise <1% of commensal microbial species (Li et al., 2019). They play an indispensable role in modulating intestinal physiology and well-being. *C. albicans* is common in humans whereas *Candida tropicalis* populates in mice. Severe dysbiosis of commensal gut fungi often results in host immune-related diseases, including intestinal bowl disease. The key role of CARD9 in controlling the DSS model of colitis-associated colorectal cancer (CAC) has been illustrated by multiple groups (Malik et al., 2018; Wang et al., 2018a). Card9 deficiency results in increased intestinal tumor mass, and the modes of how CARD9 activation in myeloid cells affects CAC could be illustrated from two aspects. Malik et al. (Malik et al., 2018) suggest that fungi-induced Card9 activation inhibits tumor growth by controlling inflammasome caspase 1 activation and subsequent IL-18 maturation in the colon during colitis and colon cancer. Since IL-18 promoted epithelial barrier restitution and IFN-γ production by inducing intestinal $CD8^+$ T cells activation, exogenous supplementation of IL-18 during DSS administration improves the host outcome in *Card9*-deficient mice, including relieving colitis symptom

and reducing tumor incidence. Pretreatment with anti-fungal agents depleted the gut commensal fungal species and exacerbated colitis and CRC. This proposes that commensal fungi might be responsible for tumor-suppressive signaling events via promotion of Card9-dependent inflammasome activation. In contrast, Wang et al. showed that Card9 controls *C. tropicalis* replication in murine macrophages. *C. tropicalis* induces differentiation of immunosuppressive myeloid-derived suppressor cells (MDSCs), which in turn suppress T cell activation. Colonization of germ-free mice with *C. tropicalis* enhances tumor mass, demonstrating the tumor-promoting properties of this fungal species (Wang et al., 2018b). These studies support an important contribution of commensal fungi to CRC and Card9-associated signaling cascades in controlling intestinal tumor progression.

Liu et al. recently provide insights to uncover the link between the fungal microbiome and lung cancer (Liu et al., 2023). The researchers utilized multi-omics techniques to analyze the species and distribution of fungal microbiome in lung adenocarcinoma (LUAD) patients. They uncovered an enrichment of the fungus *Aspergillus sydowii* within tumors of LUAD patients. By employing a LUAD murine model, they found that tumors exposed to *A. sydowii* treatment have an increased fungal burden, which is in line with findings from patient samples. The *A. sydowii* cell wall component β-glucan was further proved to be able to induce the Dectin-1/CARD9 signaling pathway and result in increased IL-1β secretion. The elevated IL-1β secretion promotes the recruitment and activation of MDSCs from bone marrow, and MDSCs was found to suppress T cells killing LUAD cells *in vitro*, as well as driving the polarization of primary $CD4^+$ T cells into regulatory T cells (Tregs).

It is unequivocally that the CLRs/CARD9 axis participates in fungal-developed tumor progression, while CARD9 also gets involved in several inflammatory diseases such as inflammatory bowl disease (IBD) and allergic bronchopulmonary aspergillosis (ABPA), a hypersensitive reaction to fungal *Aspergillus* species. Most importantly, diverse single-nucleotide polymorphisms (SNPs) in the human CARD9 gene have been reported to clinically relate to these diseases. For instance, a missense mutant of CARD9, SNP rs4077515, which carries an asparagine mutation at position 12 ($CARD9^{S12N}$), associates with both IBD and ABPA diseases (McGovern et al., 2010; Xu et al., 2018). Xu et al. uncovered the mechanism of SNP rs4077515 in CARD9 causing ABPA by utilizing knock-in mice with $CARD9^{S12N}$-encoding alleles. They demonstrated that $CARD9^{S12N}$ causes abnormal activation of RelB and excessive production of IL-5 responding to A. fumigatus. This skews T cell responses toward $T_H2$ differentiation, and the $CARD9^{S12N}$ mice displayed an allergic pulmonary response to fungal infection.

Programmed death ligand 1 (PD-L1) has been widely considered as a type I transmembrane protein that interacts with its receptor, programmed cell death 1 (PD-1). This binding induces T cell de-activation and the following immune escape. During *C. albicans* infection, PD-L1 has been found to express on T cells and natural killer cells, and immunotherapy with anti-PD-L1 antibodies relieves sepsis-induced immunosuppression and improves survival during systemic *C. albicans* infections (Kamimura et al., 2019). Yu et al. discovered that during *C. albicans* infection, the expression of PD-L1 on murine and human neutrophils is enhanced upon fungal cell wall ligand β-glucan engagement (Yu et al., 2022). PD-L1 governs neutrophil mobilization by regulating secretion of CXCL1 and CXCL2 during *C. albicans* infection. Neutrophil-specific PD-L1 deficiency impairs CXCL1/2 secretion in bone marrow but not in kidney and serum, therefore promotes neutrophil migration from germinal center bone marrow to the peripheral circulation. PD-L1 performs this negative regulation in anti-fungal immunity independent of binding with PD-1, other than the canonical interaction with PD-1 during tumor progression. Moreover, mice receiving PD-L1 blocking antibody manifest improved neutrophil-based outcomes against *C. albicans* sepsis, exhibiting increase of neutrophil infiltration into the kidney instead of accumulation in bone marrow.

## 5. Conclusion

Altogether, in recent years we have witnessed a significant improvement in understanding the cellular and molecular mechanisms of host anti-fungal immunity. A much deeper comprehension has been obtained as to the signaling transduction pathways that are responsible for activation of host defenses. The continuing discovery of the key molecules involved in the pathways, especially modulation of their activity further impacting anti-fungal responses has greatly improved our knowledge in terms of developing immunotherapy against fungal-induced sepsis. Another exciting improvement is the ongoing appreciation of the complex interplay between mycobiota and human diseases. The human mycobiome has been suggested to be implicated in carcinogenesis and numerous studies have demonstrated an important but sometimes controversy roles of CLR-mediated signal pathways in controlling disease progression. Further research is needed by using multi-omics methodology to examine the carcinogenic potential of fungi and the associated signal pathways to provide a theoretical basis for cancer control.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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