Manuscript-23070204 - R1

by xuanhuai sheng

Submission date: 29-Aug-2023 02:55PM (UTC+0800)
Submission ID: 1697392153
File name: Manuscript-23070204_-_R1.docx (170.55K)
Word count: 4201
Character count: 26265

Roles of post-translational modifications of CLR-induced signaling cascades in innate immune responses against *C.albicans*

Abstract

Candida albicans (C. albicans), a conditionally pathogenic fungus, is widespread in nature and can live in symbiosis with organisms in small quantities. When the normal microflora is imbalanced, the epithelial barrier is disrupted or the immune system becomes dysfunctional, *C.albicans* can change from commensal to pathogenic pathogen, causing both superficial and life-threatening systemic infections with no effective treatment. Perioperative patients have a higher prevalence and mortality of invasive *Candida* infections due to chronic underlying diseases, immune deficiencies and pathophysiological disorders. C-type lectin receptors (CLRs) are the main pattern-recognition receptors (PRRs) for fungal activation of innate immunity and host defence. Upon binding to ligands, CLRs induce multiple signal transduction cascades followed by activation of nuclear factor kappa B (NF-κB) through SYK- and CARD9-dependent pathways. Resolving the effects of regulatory CLR-induced signaling cascades on host immune cells is critical for understanding the molecular mechanism in regulating antifungal immunity. As one of the core factors in host innate immune regulation, protein post-translational modifications (PTMs) regulate the strength of immune effects by modulating protein conformation, stability, affinity, subcellular localization, etc. This makes the PTMs sites promising as potential targets for modulating antifungal immunity. In this review, progress in the study of PTMs in controlling CLR-induced signaling cascades throughout the process of innate immunity against *C.albicans* is mainly described. A better understanding of these mechanisms may aid in the identification and development of biomarkers and drug targets for invasive candidiasis.

Keywords: *C.albicans,* innate immunity, protein post-translational modifications, C-type lectin receptors

Introduction

The normal human body has a highly sophisticated innate and adaptive immune system that is naturally resistant to most fungal infections, with over 99% of *Candida albicans (C.albicans)* being cleared within the first hour of entry into the human bloodstream[1]. However, fungal infections have become a major cause of human disease due to the increasing number of immune-compromised population suffering from acquired immune deficiency syndrome (AIDS), chemotherapy for tumors, organ transplants, post-immunosuppressive therapy and advanced age[2]. Hundreds of millions of patients worldwide are infected with the pathogenic fungal each year, resulting in at least 1.5 million deaths per year, similar to the number of deaths due to tuberculosis[3]. *C.albicans* is the most common fungal pathogen, accounting for more than half of invasive

candidiasis[4].

In perioperative patients, many factors can increase the risk of *C.albicans* infection. First, operation suppresses the body's immune function by inhibiting the activity of natural killer (NK) cells within hours of the surgery and it can persistently work as inhibition[5]. NK cells are a significant component of innate immune system, play an important role in resistance to fungal infection and kill fungus by release of soluble cytotoxic molecules (perforin and granzyme) or apoptosis pathway[6, 7]. Second, perioperative pain induced by inflammatory factors can restrain immune function by interaction between the central nervous system (CNS), hypothalamic-pituitary-adrenal (HPA) axis pathways and immune system[8]. Third, critical patients who receive surgery often need central venous indwelling catheter or peripheral venous indwelling catheter. Fungus colonize in venous catheters, enter the blood and cause bloodstream infections, threatening the safety of patients' lives[9, 10]. Last, longer hospital stays and intensive care unit (ICU) stays undoubtedly greatly increase the risk of infection. Fungal infection is a momentous factor for ICU mortality[11].

Innate immunity plays a crucial role as the host's primary defense mechanism against invading pathogens, relying on intricate and dynamic interactions among various cellular and molecular components. Distinguishing between non-self pathogens and self components is a fundamental function of the innate immune system. Pattern recognition receptors (PRRs) recognize pathogen-associated molecular patterns (PAMPs). These PRRs are mainly expressed by myeloid macrophages including Toll-like receptors (TLRs), C-type lectin receptors (CLRs), nucleotide-binding oligomerization domain (NOD) like receptors (NLRs), retinoic acid-inducible gene 1 (RIG1)-like receptors, complement components and receptors[12]. All of these PRRs can mediate antifungal immunity, and the most widely studied are CLRs. The PAMPs mainly consist of *Candida* cell wall-associated glycans (mannans, glucans, chitin)[13].

CLRs are the main PRRs for fungal activation of innate immunity and host defence[14]. The receptors dectin-1, dectin-2, dectin-3 and mincle bind to ligands and then recruit spleen tyrosine kinase (SYK) intracellularly and are activated by an intermolecular auto-phosphorylation mechanism[15]. Activated SYK further activates dedicator of cytokinesis 2 (DOCK2), phospholipase C-γ2 (PLC-γ2) and protein kinase C-δ (PKC-δ) by phosphorylation, the latter phosphorylates caspase recruitment domain containing protein 9 (CARD9) and vesicles containing glucose transporter 1 (GLUT1). Phosphorylation of vesicles promotes GLUT1 translocation to the cytosol and thus increases glucose uptake by immune cells. SYK and CARD9 are transported from the cytosol to the cytoplasm via α-tubulin. Then CARD9, B cell lymphoma 10 (BCL10), and mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) form the CBM complex. This complex ultimately leads to the activation of downstream nuclear factor kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs)[16]. These signaling pathways turn on a series of effector mechanisms, including the release of proinflammatory cytokines and phagocytosis, finally leading to fungi clearance[17].

Proteins are essential components of living organisms, are the material basis of life, and are involved in practically all cellular processes. To date, more than 50% of proteins in human cells have been found to have different types of post-translational modifications (PTMs)[18]. PTMs can rapidly regulate various intracellular activities and expand the diversity of protein functions by affecting protein activity, stability, localization and signal transduction under physiological and pathological conditions[19, 20], such as classical modifications including phosphorylation, ubiqui-

tination and methylation, as well as non-classical glycosylation, acetylation, malonylation and succinylation[21]. During the anti-fungal process of innate immune cells, many proteins in the signaling pathway are required to produce more functions by way of PTMs, and these related enzymes involved in protein PTMs can indirectly and specifically regulate the anti-fungal ability of immune cells. Whether positively or negatively regulated, these modification-related enzymes have the potential to become targets for modulating the immune system. Therefore, this review focuses on how protein PTMs are involved in the innate immune response against *C.albicans*, primarily by regulating CLR-induced signaling cascades through ubiquitination, phosphorylation, acetylation and glycosylation.

Phosphorylation and dephosphorylation

In organisms, phosphorylation is the most widespread form of covalent modification in protein PTMs[22]. Phosphorylation plays an important regulatory role in the proper functioning of proteins by the transfer of the γ-position phosphate group of ATP or GTP to the amino acid residues of the substrate protein (mainly serine and threonine) catalyzed by phosphokinase, while the reverse process involves the removal of the corresponding phosphate group by protein kinase[23]. It is the opposing roles of these two enzymes and the energy consumption and production involved that make phosphorylation the preferred mode of regulation of many physiological activities in the body(e.g. transcriptional regulation, signal transduction, DNA damage repair)[24] **(Figure 1)**.

Phosphorylation

EPHB2

EPH receptor B2 (EPHB2) is a member of the EPH receptor family of receptor tyrosine kinase transmembrane glycoproteins. EPHB2 was recently found to directly recognize the fungal cell wall component β-glucan and to synergize with dectin-1 to activate downstream signaling. EPHB2 directly phosphorylates the downstream kinase SYK and promotes its activation. In *EPHB2* $^{-/-}$ macrophages, fungal-stimulated phosphorylation of SYK was almost completely lost. Ephb2-deficient mice were significantly more susceptible to *C. albicans*–induced infection than the WT mice[25]. EPHB2 also phosphorylates T-cell-activated Rho GTPase-activating protein (TAGAP) at the Y310 site, and TAGAP acts as an adapter to regulate upstream EPHB2 and downstream CARD9 signaling[26]. In summary, EPHB2 regulates dectin-mediated immune signaling by modulating the phosphorylation of SYK and TAGAP during antifungal host defense.

SYK

SYK includes two N-terminal Src Homology 2 (SH2) domains, one C-terminal kinase domain and two interdomain linkers[27]. These structural domains are bound together, leaving the

SH2 domain in a stable inactivation state[28]. Phosphorylation is the most common method to activate SYK, and there are 10 autophosphorylation sites in SYK[29]. CLRs engagement promotes Src-dependent phosphorylation of its immunoreceptor tyrosine-based activation motif (ITAM), recruits the SHP-2 tyrosine phosphatase and activates SYK[30, 31]; this binding leads to conformational changes and unfolding of SYK, which is subsequently activated by SYK's own phosphorylation or by phosphorylation of its upstream kinases[29]. SYK, which acquires enzymatic activity, activates its downstream substrate proteins through phosphorylation or even direct interaction and transduces its downstream signaling cascade. Activated SYK phosphorylates DOCK2 at tyrosine 985 and tyrosine 1405, promoting the recruitment and activation of Rac GTPase, thereby increasing reactive oxygen species (ROS) production required for macrophage signaling activation and bactericidal activity[32].

PKC-δ

Protein kinase C-δ (PKC-δ) is activated under dectin-SYK signaling, mediates CARD9 phosphorylation at Thr231, and is involved in CBM complex assembly and classical NF-κB regulation. Defective innate response of *PKC-δ*$^{-/-}$ dendritic cells to dectin-1, dectin-2, or mincle stimulation, while dendritic cells lacking PKC-α, PKC-β, or PKC-θ are immunocompetent. In addition, *C.albicans* induced a significant reduction in the production of cytokines such as TNF, IL-10 and IL-2 in *PKC-δ*$^{-/-}$ cells compared to wild type. Similarly, *RKC-δ*$^{-/-}$ mice are highly susceptible to fungal infections. Thus, PKC-δ is an important link between SYK activation and CARD9 signaling in CLR-mediated natural immunity and host protection[33]. On the other hand, PKC-δ promotes GLUT1 insertion into the cell membrane by phosphorylating vesicles bearing GLUT1. This promotes glucose uptake by neutrophils thereby enhancing the neutrophil metabolic fitness[34].

Dephosphorylation

STS

The steroid sulfatase (STS) proteins are homologous protein phosphatases that share overlapping functions[35], and are negative regulators of multiple signaling pathways. For example, in T cells, STS phosphatase helps set the threshold for T cell activation by targeting Zap-70, an important kinase downstream of the T cell receptor[36]. In addition, STS-1 has been shown to control signaling downstream of GPVI-FcRγ in platelets and FcεR in mast cells by targeting the ZAP-70 homolog SYK[37]. At 12 to 18 h after invasive *Candida* infection, the fungal burden in the kidneys of *STS*$^{-/-}$ mice began to decrease compared with WT, and a large number of leukocytes in *STS*$^{-/-}$ mice entered the kidneys to fight the infection, improving the survival rate of the mice. After stimulation of BMDCs with fungal ligands, we observed no difference in other antifungal responses, such as cytokine or nitric oxide production, by *STS*$^{-/-}$BMDCs, but were able to enhance ROS production. Although the signaling pathway from dectin to the initiation of the ROS response has not been fully elucidated, the involvement of SYK kinase has been identified[38]. Hyperphos-

phorylation of SYK in *STS*$^{-/-}$BMDCs was observed using a phosphospecific antibody that recognizes the activation of SYK tyrosine phosphorylation. STS may be involved in antifungal immunity by regulating the level of SYK phosphorylation and activation, and thus ROS production[39].

PP1

Recruitment of protein phosphatase 1 (PP1) to CARD9 keeps CARD9 in a dephosphorylated, self-inhibited state, thereby inhibiting CBM complex formation and suppressing downstream NF-κB and JNK signaling. PP1 does not have a direct effect on CARD9 and requires the mediation of downstream of kinase 3 (DOK3). DOK3 is a junctional molecule preferentially expressed in hematopoietic cells[40]. It acts as a specific regulator downstream of several immune receptors, including TLR3, TLR4 and BCR, during viral infection, endotoxin stimulation and plasma cell differentiation[41-43]. In invasive fungal infections, DOK3 acts as an intermediate molecule that promotes the recruitment of PP1 to CARD9. Deletion of DOK3 enhances various antifungal effector functions of neutrophils, including phagocytosis, extracellular trap network and pro-inflammatory cytokine production, through dephosphorylation of CARD9 by PP1, thereby increasing the fungicidal activity of neutrophils[44]. PKC-δ and PP1 keep CARD9 in a balanced activation state through phosphorylation and dephosphorylation to avoid excessive inflammatory immune response and immunosuppression **(Figure 2)**.

Ubiquitination and Deubiquitination

Ubiquitin (Ub) is a protein consisting of 76 amino acids, and target proteins can be modified by monoubiquitination or polyubiquitination[45]. A single ubiquitin molecule is repeatedly attached to a ubiquitin lysine residue to form a ubiquitin chain. The ubiquitin molecule itself contains seven lysine residues, all of which can be ubiquitinated, and the N-terminus can also be attached to ubiquitin to form eight types of ubiquitin chains (K6, K11, K27, K29, K33, K48, K63 and MET1)[46]. Ubiquitination, an important PTM modality, is the process of ubiquitin binding to substrate molecules catalyzed by the ubiquitin-proteasome system (UPS). This process is facilitated by three enzymes: ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3). E1 activates ubiquitin, E2 transfers ubiquitin to E3, and finally, E3 catalyzes the covalent attachment of ubiquitin to the target protein, or in some cases, E3 directly adds ubiquitin from E2 to the substrate[47]. Of these, E3 determines the specificity and precision of ubiquitin and substrate attachment, and the human genome has approximately more than 600 E3s, a large number compared to the 2 E1s and approximately 40 E2s. Thus ubiquitination is more complex and functionally diverse than other protein modifications[48]. E3 ubiquitin ligases are divided into three families: the RING finger-type, HECT-type and RING-between-RING (RBR)[49]. At the same time, the human genome also has about 100 deubiquitinases (DUB), classified into 7 classes: USP, UCH, OUT, MJD, JAMM, MCPIP, MINDY, which are also highly specific and responsible for removing ubiquitin chains from proteins and other molecules[50]（**Figure 3**）.

Ubiquitination

CBL-B

Casitas B-lineage lymphoma protein b (CBL-B) is one of three homologous proteins of the Cbl E3 ubiquitin ligase family that is ubiquitously expressed in all leukocyte subpopulations and negatively regulates the activation of signaling pathways such as T-cell antigen receptor (TCR), B-cell antigen receptor (BCR), CD28, Toll-like receptor 4 (TLR4), high-affinity IgE receptor (FcεR1), and epidermal growth factor receptor[51-55]. During invasive *C.albicans* infection, *CBL-B*$^{-/-}$ mice are highly resistant to disseminated candidiasis, with resistance characterized by reduced weight loss following infection, an absence of inflammatory damage, and enhanced survival. Enhanced fungal clearance in *CBL-B*$^{-/-}$ mice within 24-48 hours of bloodstream infection, 10-fold reduction in fungal load in different peripheral organs compared to control group[56]. *CBL-B*$^{-/-}$ bone marrow-derived myeloid mononuclear macrophages with reduced ligand-mediated receptor internalization and degradation, increased dectin receptor expression, and increased production of various pro-inflammatory factors and ROS. This is because CBL-B can mediate the ubiquitination of these activated CLRs through the mutual binding of the junction protein FcR-γ and the tyrosine kinase SYK. Dectin-1 K2, K27, and K34, dectin-2 K10 and K12, dectin-3 K9 are the sites of ubiquitination of dectin-1, -2, -3 respectively[57, 58], and the ubiquitinated CLRs are sorted into lysosomes by the transport-essential endosomal sorting complex (ESCRT) system for degradation, thereby negatively regulating the CLR-mediated innate immune response against fungal infections.

C-CBL

CBL-B homologous family member c-Casitas B-lineage lymphoma (C-CBL) modulates intestinal inflammation by inhibiting fungal-induced non-classical NF-κB activation. Intestinal fungal-derived mannan activates C-CBL in dendritic cells (DCs) via dectin-2 and dectin-3, thereby promoting C-CBL-mediated ubiquitination and degradation of RelB, a non-classical NF-κB family member. Meanwhile, the classical NF-κB family member p65 mediates the transcription of the anti-inflammatory cytokine IL-10 gene to suppress colitis, and RelB binds to p65 and inhibits p65-mediated production of IL-10. Thus, the lack of C-CBL in DCs promotes mannan-induced activation of RelB, which binds to p65, thereby inhibiting p65-mediated IL-10 transcription, decreasing anti-inflammatory cytokines, and over-immunization, making mice more susceptible to Dextran Sulfate Sodium Salt (DSS)-induced colitis[59].

TRIM31

Up to now, nearly 80 TRIM family proteins have been identified in the human genome, many of which have been shown to have E3 ubiquitin ligase activity and are involved in various life activities of the organism[60]. For example, tripartite motif containing 31 (TRIM31), a member of

the TRIM/RBCC family, is a RING finger E3 ubiquitin ligase that is involved in the regulation of viral and fungal infections, non-alcoholic fatty liver disease, hypertensive nephropathy, ischemic brain injury and other diseases[61-65]. TRIM31 also binds directly to NOD-like receptor thermal protein domain associated protein 3 (NLRP3), and K48-linked ubiquitination mediates protein degradation of NLRP3. TRIM31 can limit NLRP3 inflammasome activation under physiological conditions and is therefore expected to be a potential therapeutic target for NLRP3 inflammasome related diseases[66]. Recently, TRIM31 was found to be a key regulator of SYK activation. RIM31 interacts with SYK and catalyzes K27-linked polyubiquitination at the Lys375 and Lys517 sites of SYK, thereby promoting plasma membrane translocation of SYK and binding to CLRs, and also inhibits dephosphorylation of SYK by the phosphatase SHP-1, which in turn enhances SYK-mediated downstream signaling (CBM complex, inflammasome formation). In vitro TRIM31 deficient BMDCs and BMDMs significantly reduced the production of pro-inflammatory cytokines such as IL-6, TNF-α, IL-1β, IL-12, and IL-23. Similarly, *TRIM31*$^{-/-}$ mice are more susceptible to fungal infection, have a more severe renal inflammatory response, and have lower survival rates than *TRIM31*$^{+/+}$ mice[62].

TRIM62

CARD9 is a central component of natural immune signaling against fungi mediated by CLRs, and when not activated, CARD9 is in a self-inhibited state. TRIM62 mediates polyubiquitination of CARD9 at the K27-linked at the Lys125 site of CARD9, disrupting the inhibitory state of CARD9 and enhancing the transduction of signaling pathways in which CARD9 is involved. Thus, similar to *CARD9*$^{-/-}$ mice, *TRIM62*$^{-/-}$ mice have an increased susceptibility to fungal infections[67]. Genetic sequencing of patients with inflammatory bowel disease (IBD) shows that the protective CARD9 variants are not ubiquitinated by TRIM62 and suggests that the protective effect of C-terminal truncation may be mediated by the loss of TRIM62 interactions, thereby limiting the pro-inflammatory cytokine response. If the CARD9-TRIM62 interaction is blocked, the inflammatory response can be limited, and the development of small-molecule inhibitors that block the CARD9-TRIM62 interaction have been developed that might be useful for IBD treatment[68].

NEDD4

Neuronal precursor cell-expressed developmentally down-regulated 4 (NEDD4) is a HECT type E3 ubiquitin ligase that has been shown to positively regulate T cell activation and proliferation[69]. Additionally, it has also been reported that Nedd4 is involved in anti-cellular bacterial clearance by promoting autophagy[70]. *Nedd4*$^{-/-}$ mice are highly susceptible to systemic *C.albicans* infection, which is associated with increased organ fungal load, defective inflammatory response, impaired recruitment of leukocytes to the kidney, and impaired granulocyte expression of ROS. The specific regulatory mechanism of Nedd4 is unclear, at the molecular level, *Nedd4*$^{-/-}$ macrophages showed reduced TGFβ-activated kinase 1 (TAK-1) and NF-κB activity and normal SYK and PKC-δ activity during *C.albicans* infection. This indicates that NEDD4 regulates signaling events downstream of PKC-δ and upstream of TAK-1 to enhance immune cell killing against

C.albicans[71].

Deubiquitination

UPS15

The ubiquitin-specific proteases (USPs) family comprises the largest number of deubiquitinating enzymes (DUBs). During antifungal immunization, USP15 removes TRIM62-mediated ubiquitination of CARD9 and inhibits the activation of CARD9, thereby inhibiting the formation of the CBM complex. Thus the activation of CARD9 is co-regulated by USP15 and TRIM62 so that the degree of activation is in a state of equilibrium[72].

OTUD1

Ovarian tumor deubiquitinase 1 (OTUD1) is also an important regulator of CARD9. OTUD1 mainly removes K29, K33 and K63-linked polyubiquitination from CARD9 and promotes the formation of the CBM complex. OTUD1 deficiency reduces CARD9-mediated signaling, leading to reduced production of pro-inflammatory cytokines and chemokines. Meanwhile, *OTUD1*$^{-/-}$ mice showed increased susceptibility to fungal infections[73]. In other studies OTUD1 exerts tumor suppression by removing K63-linked ubiquitination on Yes-associated protein (YAP) and inhibiting the degradation of P53[74]. In addition, OTUD1 negatively regulates the RNA virus signaling pathway by targeting smad ubiquitination regulatory factor 1 (Smurf1)[75] **(Figure 4)**.

Acetylation and Deacetylation

Lysine acetylation is a conserved PTM, most commonly histone acetylation, and is usually regulated by acetyltransferase (KAT) and deacetyltransferase (KDAT)[76]. Acetylation modifications usually occur in the structural domains of proteins, such as α-helix and β-fold, and the proteins with acetylation modifications are usually conserved, such as metabolism-related enzymes, ribosomes and molecular chaperones[77]. With the continuous research and development on protein acetylation, existing studies have demonstrated that acetylation also plays an important regulatory role in the antifungal immune process.

Acetylation

αTAT1

AP2A1 and α-tubulin N-acetyltransferase 1 (αTAT1) are recruited to α-tubulin via Myosin 1F (MYO1F), which promotes acetylation of α-tubulin; SYK and CARD9 molecules then activate downstream signaling pathways through acetylated tubules from the cell membrane into the cytoplasm. MYO1F is an unconventional myosin expressed mainly in mammalian immune cells that regulates the migration of mast cells and neutrophils[78, 79], and also regulates M1 polarization by stimulating intercellular adhesion of macrophages[80]. MYO1F also plays a key role in the activation of antifungal natural immune signaling and is required for dectin-induced acetylation of α-tubulin. Following systemic *Candida* infection, MYO1F-deficient mice are more severely infected relative to wild-type mice. Administration of AGK2 or AK-1, inhibitors of the deacetylase SIRT2, promoted increased dectin-activated signaling and proinflammatory gene expression and produced a protective effect in mice with systemic *Candida* infection[81].

Deacetylation

HDAC11

Histone deacetylases (HDACs) are enzymes that catalyze the removal of acetyl functional groups from the lysine residues of both histone and nonhistone proteins[82]. Based on structure and function HDACs are classified into four groups: class I HDACs (HDAC1, 2, 3 and 8), class II HDACs (HDAC4, 5, 6, 7, 9 and 10), class III HDACs (SIRT1–SIRT7) and class IV HDAC (HDAC11)[83]. As the only class IV HDAC, HDAC11 plays a critical role in a variety of cellular events including cell proliferation and differentiation, metabolism and tumorigenesis[84-86]. Loss of HDAC11 increases histone 3 and 4 acetylation at the *Nos2* promoter and leads to enhanced *Nos2* transcription and corresponding iNOS levels in macrophages. The transcriptional repressor of *Nos2*, signal transducer and activator of transcription 3 (STAT3), interacts with HDAC11 and acts as a scaffolding protein to facilitate the binding of HDAC11 to the *Nos2* promoter. Inhibition of STAT3 significantly reduced the aggregation of HDAC11 on the *Nos2* promoter. Similarly, HDAC11 deletion reduced the abundance of STAT3 binding on the *Nos2* promoter. Thus fungal pathogens stimulate *HDAC*$^{-/-}$ mouse macrophages to produce more NO and ROS. HDAC11 inhibitor FT895 shows antifungal therapeutic effects in both *C.albicans* infected mice and human cells[87] **(Figure 5)**.

Glycosylation

In the human immune system, all immunoglobulins and most complement components are glycosylated, and depending on the glycosidic bond. there are two main types of glycosylation: O-linked glycosylation and N-linked glycosylation[88]. Through glycosyltransferases, sugars form glycosidic bonds with amino acid residues of various proteins to form glycoproteins, which play an important role in the regulation of protein folding and stabilization, cell growth, receptor activation, cell adhesion and immune response[89, 90].

JAGN1

The protein encoded by *Jagunal homolog 1* (*JAGN1*) is an endoplasmic reticulum transmembrane protein that functions in the early secretory pathway and is required for neutrophil differentiation and survival. *JAGN1* mutations can cause severe congenital neutropenia[91]. Following systemic *C.albicans* infection, *JAGN1*-deficient mice showed significant weight loss, enlarged mortality and increased organ fungal burden. Deletion of JAGN1 alters glycoprotein processing and protein transport in the endoplasmic reticulum and Golgi by analysis of mass spectrometry data. In particular, altered glycosylation of the cell adhesion and migration molecules CD177, CD11b and CD18 was observed as well as significant alterations in the glycosylation of neutrophil collagenase (Mmp8), matrix metalloproteinase-9 (Mmp9), lactoferrin (Ltf), lipocalin 2 (Lcn2), binding bead protein (Hp) and myeloid bacteriocin (F1), which are associated with cytotoxic effector functions of neutrophils. Thus, deletion of JAGN1 leads to obvious glycosylation changes in key molecules involved in neutrophil migration and neutrophil cytotoxicity[92].

Discussion

Invasive *Candida* infections are a serious threat to human health, especially in perioperative immunocompromised patients. For invasive *Candida* infections, the main clinical treatment is still the use of antifungal drugs. There are three main classes of antifungal drugs in common use, namely azoles, polyenes and echinocandins[93]. These antifungal drugs usually target the ergosterol biosynthetic pathway, fungal cell membranes or cell walls. However, the increasing toxicity and resistance of these drugs has led to unsatisfactory clinical outcomes, and the mortality rate of invasive *Candida* infections still exceeds 50%[94]. Therefore, it is necessary to explore novel therapeutic approaches to combat fungal infections.

The regression of any infection depends on the pathogenic capacity of the pathogenic bacteria and the ability of the immune system to kill it. In addition to developing new drugs to target *Candida* itself, it is possible to fight infection by modulating the immune system. Through the previous description of the regulatory role of the protein PTMs in the antifungal immune process, we have learned about the targets and mechanisms of action of the enzymes associated with PTMs in the antifungal immune process **(Table 1)**. These substances play a very important role in the

control of fungal infections. Therefore, modulating the progression of anti-fungal immunity through the regulation of protein PTMs represents a promising avenue for intervention.

In existing studies, systemic in vivo delivery of CBL-B siRNA into mice protects them from lethal disseminated candidiasis and greatly reduced post-infection mortality and organ fungal burden. Administration of AGK2 or AK-1 inhibitors of the deacetylase SIRT2 during *Candida* albicans stimulation of immune cells, promoted increased dectin-activated signaling and proinflammatory gene expression and produced a protective effect in mice with systemic *Candida* infection[57, 87]. The above approaches interfere with enzymes, negatively regulated in the immune process against *Candida* albicans, by siRNA and inhibitors, thus enhancing the antibacterial capacity of the cells, with the same effect in mice. This suggests that the anti-inflammatory capacity of immune cells can be directly regulated by regulating the activity of these enzymes. This allows these enzymes to function as potential therapeutic targets, and the immunity of the host during invasive *Candida* infections can be enhanced by developing drugs that target these enzymes.

In conclusion, PTMs modulating host innate immune response against *C.albicans* invasion have great research value, however, it still has a long way to go to provide clinical treatment for fungal infections, and more sustained and in-depth research is needed in the future.

Manuscript-23070204 - R1

ORIGINALITY REPORT

30%	22%	24%	6%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.ncbi.nlm.nih.gov Internet Source	4%
2	www.nature.com Internet Source	2%
3	www.researchgate.net Internet Source	2%
4	www.frontiersin.org Internet Source	2%
5	Submitted to The University of Manchester Student Paper	2%
6	www.science.gov Internet Source	1%
7	www.jci.org Internet Source	1%
8	Xiaorong Chen, Honghai Zhang, Xueer Wang, Zhugui Shao et al. "OTUD1 Regulates Antifungal Innate Immunity through Deubiquitination of CARD9", The Journal of Immunology, 2021 Publication	1%

9	David Frank, Shamoon Naseem, Gian Luigi Russo, Cindy Li, Kaustubh Parashar, James B. Konopka, Nick Carpino. " Phagocytes from Mice Lacking the Sts Phosphatases Have an Enhanced Antifungal Response to ", mBio, 2018 Publication	1 %
10	Dominikus Strasser, Konstantin Neumann, Hanna Bergmann, MohlopheniJ. Marakalala et al. "Syk Kinase-Coupled C-type Lectin Receptors Engage Protein Kinase C-δ to Elicit Card9 Adaptor-Mediated Innate Immunity", Immunity, 2012 Publication	1 %
11	Jie-Lin Duan, Hui-Qian He, Yao Yu, Tao Liu et al. "E3 ligase c-Cbl regulates intestinal inflammation through suppressing fungi-induced noncanonical NF-κB activation", Science Advances, 2021 Publication	1 %
12	Yi Teng, Yuhang Chen, Xinyi Tang, Shengjun Wang, Kai Yin. "PAD2: A potential target for tumor therapy", Biochimica et Biophysica Acta (BBA) - Reviews on Cancer, 2023 Publication	1 %
13	www.focisnet.org Internet Source	1 %

14	L. Jia, Y. Sun. "SCF E3 Ubiquitin Ligases as Anticancer Targets", Current Cancer Drug Targets, 2011 Publication	1 %
15	pubmed.ncbi.nlm.nih.gov Internet Source	1 %
16	www.mdpi.com Internet Source	1 %
17	hdl.handle.net Internet Source	<1 %
18	www.jimmunol.org Internet Source	<1 %
19	Submitted to University of Birmingham Student Paper	<1 %
20	Jia Tong Loh, Shengli Xu, Jian Xin Huo, Susana Soo-Yeon Kim, Yue Wang, Kong-Peng Lam. "Dok3–protein phosphatase 1 interaction attenuates Card9 signaling and neutrophil-dependent antifungal immunity", Journal of Clinical Investigation, 2019 Publication	<1 %
21	Submitted to Queen Mary and Westfield College Student Paper	<1 %
22	www.annualreviews.org Internet Source	<1 %

23	Long, Xin, Yang Yu, Laszlo Perlaky, Tsz-Kwong Man, and Michele S. Redell. "Stromal CYR61 Confers Resistance to Mitoxantrone via Spleen Tyrosine Kinase Activation in Human Acute Myeloid Leukaemia", British Journal of Haematology, 2015. Publication	<1 %
24	Zoi Alexopoulou, Johannes Lang, Rebecca M. Perrett, Myriam Elschami et al. "Deubiquitinase Usp8 regulates α-synuclein clearance and modifies its toxicity in Lewy body disease", Proceedings of the National Academy of Sciences, 2016 Publication	<1 %
25	coek.info Internet Source	<1 %
26	Submitted to Cranfield University Student Paper	<1 %
27	worldwidescience.org Internet Source	<1 %
28	Wanwei Sun, Heping Wang, Huijun Hu, Xiaojian Ma et al. "Cutting Edge: EPHB2 Is a Coreceptor for Fungal Recognition and Phosphorylation of Syk in the Dectin-1 Signaling Pathway", The Journal of Immunology, 2021 Publication	<1 %

29	Renata Z. Jurkowska, Albert Jeltsch. "Genomic Imprinting-The Struggle of the Genders at the Molecular Level", Angewandte Chemie International Edition, 2013 Publication	<1 %
30	Thomas Timm, Christiane Hild, Gerhard Liebisch, Markus Rickert, Guenter Lochnit, Juergen Steinmeyer. "Functional Characterization of Lysophospholipids by Proteomic and Lipidomic Analysis of Fibroblast-like Synoviocytes", Cells, 2023 Publication	<1 %
31	aapep.bocsci.com Internet Source	<1 %
32	patents.google.com Internet Source	<1 %
33	www.e-sciencecentral.org Internet Source	<1 %
34	cyberleninka.org Internet Source	<1 %
35	mdpi-res.com Internet Source	<1 %
36	Hui Song, Chunyuan Zhao, Zhongxia Yu, Qizhao Li, Rongzhen Yan, Ying Qin, Mutian Jia, Wei Zhao. "UAF1 deubiquitinase complexes facilitate NLRP3 inflammasome activation by	<1 %

promoting NLRP3 expression", Nature Communications, 2020
Publication

37	www.dovepress.com Internet Source	<1%
38	Han Wu, Xiaofan Yin, Xibao Zhao, Zherui Wu, Yue Xiao, Qianqian Di, Ping Sun, Haimei Tang, Jiazheng Quan, Weilin Chen. "HDAC11 negatively regulates antifungal immunity by inhibiting Nos2 expression via binding with transcriptional repressor STAT3", Redox Biology, 2022 Publication	<1%
39	akjournals.com Internet Source	<1%
40	pdfcoffee.com Internet Source	<1%
41	portlandpress.com Internet Source	<1%
42	www.omicsdi.org Internet Source	<1%
43	Rebeaud, F.. "Dlgh1 and Carma1 MAGUK proteins contribute to signal specificity downstream of TCR activation", Trends in Immunology, 200705 Publication	<1%

44	rupress.org Internet Source	<1%
45	Nick Carpino, Shamoon Naseem, David M. Frank, James B. Konopka. "Modulating Host Signaling Pathways to Promote Resistance to Infection by Candida albicans", Frontiers in Cellular and Infection Microbiology, 2017 Publication	<1%
46	Neil A. R. Gow. "An integrated model of the recognition of Candida albicans by the innate immune system", Nature Reviews Microbiology, 01/2008 Publication	<1%
47	Patrick K. Nuro-Gyina, Na Tang, Hui Guo, Chengkai Yan, Qiuming Zeng, Thomas J. Waldschmidt, Jian Zhang. "HECT E3 Ubiquitin Ligase Nedd4 Is Required for Antifungal Innate Immunity", The Journal of Immunology, 2021 Publication	<1%
48	Zhibin Wu, Yunyuan Huang, Ke Liu, Jinrong Min. "N/C-degron pathways and inhibitor development for PROTAC applications", Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms, 2023 Publication	<1%

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	Off		