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Protein kinases as potential anticandidal drug targets

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1. ABSTRACT

Candidal infections are increasing at an alarming rate due to hospital acquired infections causing high mortality rates worldwide. Moreover, the emergence of drug resistant *Candida* strains is the major impediment against effective therapeutics. Thus, there is an imperious need to search for novel antifungal drug targets. Among various fungi, *Candida albicans* is one of the most prevalent human fungal pathogen. Protein kinases modify other signaling molecules through phosphorylation and transduce extracellular stimuli for adaptation ensuing *C. albicans* growth, persistence and pathogenesis. In *C. albicans*, there are various kinds of kinases such as MAP (Mitogen Activated Protein) kinase cascade involving Hog1 (High-osmolarity glycerol) and Cek1 (*C. albicans* ERK-like Kinase1) mediated pathways, cyclin dependent pathway, cAMP (cyclic adenosine monophosphate) -dependent protein kinase pathway and TOR signaling pathway. Herein we have reviewed the variety of functions served by protein kinases in *C. albicans*. Additionally, we have discussed the inhibitors for targeting these kinases. Together, we explore the potential of these kinases as effective drug target and discuss the progress made in the development of inhibitors against these targets.

2. INTRODUCTION

Candida albicans is among the most prevalent human fungal pathogen causing candidal infections predominantly in immunocompromised patients (1, 2, 3). The incidence of increasing resistance to antifungals by *C. albicans*, has reached an alarming rate and poses a formidable challenge in front of the scientific community. They exist in basically two forms namely yeast and hyphal form where the later is mainly required for invasion into host tissues along with other factors like phospholipases and secretary enzymes (4, 5). The emergence of resistance by a phenomenon known as multidrug resistance (MDR) in yeast, has rendered most of the currently available drugs less effective (6). Therefore, an understanding of the mechanisms of antifungal resistance at the molecular level is indispensable, for the development of strategies to circumvent MDR in this pathogenic yeast. The signaling cascades of yeasts involved in their survival, persistence, and pathogenesis, represent a plethora of diverse entities, which are yet to be exploited. The signaling molecules are mandatory to play crucial roles in important pathways and regulation of virulence traits (7). *C. albicans* adapts and activates signaling pathways which makes it able to survive hostile environment within the host cell. One such signaling molecule which has an immense significance and governs major cellular responses is protein kinase.

Protein kinases are one of the most widely studied signaling molecules that constitute approximately 2% in the eukaryotic genome (8). They are enzymes which can catalyze the phosphotransfer reactions. It has been reported that only few kinases are constitutively active as the unregulated activity of the protein kinases could be deleterious or lethal to cells. The evolutionary studies have shown that the activity of several protein kinases are precisely controlled by various mechanisms for the fine- tuning of functions occurring in cells (8). Yeast model is widely used for studying the function and types of protein kinases. It has been reported that yeast genome encodes around 117 protein kinases (9). The regulation of protein kinases involves the phosphotransferases which can catalyze gamma phosphate transfer from a nucleoside triphosphate to the hydroxyl groups which are present in the threonine, serine, and tyrosine side chains of proteins. The activity of protein kinases has been directed by activation of T-loops in the protein. The mechanisms of regulation of protein kinases involve the phosphorylation, dephosphorylation, binding of ligands, accumulation, and localization of signaling molecules (10). The present review focuses on discussing various kinases in *C. albicans* and its potential as a target for efficient antifungal therapeutics. This review also focuses on potential inhibitors of these kinase pathways which can cause a disturbance in coordination and sequence of events occurring in kinase pathways, hence could be applied as a vital therapeutic approach.

3. PROTEIN KINASES IN *C. ALBICANS*

Protein kinases in *C. albicans* have been discussed below:

3.1. MAP (mitogen activated protein) kinase cascade

Mitogen-activated protein kinases (MAPK) or Extracellular signal-regulated protein kinase (EPK) is involved in the activation of transcription factors which play important role in cell cycle regulation, hyphal development, stress responses and changes in glycosylation, etc (11). They are serine/threonine or tyrosine specific kinases which get phosphorylated upon activation. It is regulated by a coordinated sequence of the phosphorylation events (12). They function in signaling cascade which is activated by ligand binding site lying on the cell surface receptor which in turn activates many MAPK/EPK kinases that phospholyrates the substrates (13). Generally, it comprises of three modules involving the MAPKKK (MAP kinase kinase kinase) which becomes phosphorylated on the advent of upstream signaling, which in turn phosphorylates the MAPKK (MAP kinase kinase) and, passes on the signal to the MAPK. A final stimulus is again perceived by the transcription factors which gives a necessary response. The most well-studied MAP kinase mediated pathways are HOG pathway, CEK mediated pathway and cell wall integrity pathway (Table 1). All these pathways have well- defined roles in *C. albicans* commonly serving in the morphogenesis but at the same time are required under distinct conditions also to be activated by different stimuli (14, 15).

3.1.1. HOG pathway (high-osmolarity glycerol)

This pathway comes into play when there is a high osmolarity or low water activity. It was well studied in eukaryotes but later also was elucidated in lower eukaryotes. In *Saccharomyces cerevisiae*, it is very well studied, in a series of reactions that occurs under high osmolarity and triggering the accumulation of glycerol which acts as an intracellular osmolyte (16). *HOG1* gene encodes for MAPK Hog1p. There is involvement of two cascades which includes Ypd1p, Sln1p, Ssk1p, and Ssk2p/Ssk22p kinases. Another putative membrane protein Sho1p activates the Pbs2p MAP kinase through Ste11p. The signals converge at the Pbs2p level and activates by phosphorylating the Hog1 pathway (17). In *C. albicans*, the signal activates the two-component protein comprising Sln1p and Ypd1p and Ssk1p phosphorelay proteins. Under normal isotonic conditions, there is constitutive activation of a three-protein branch which phosphorylates Ssk1p and blocks activation of the downstream cascade (18). However, under high osmolarity conditions, this whole cascade is reversed. The dephosphorylation of Ssk1p initiates the activation of the Ssk2p/Ssk22p proteins (19). Another cascade starts through the association of Ste11 MAPKKK and Ste11-interacting protein Ste50, which in turn binds to the Ste20 p21-activated kinase (PAK), activating the small GTPase, Cdc42 (Cell division control) protein and the transmembrane Sho1p adaptor protein. Now, these signals converge at MAPKK Pbs2p level, which can activate the Hog1 MAPK by phosphorylation, finally leading to downstream effectors (20). Activation of *HOG1* genes leads to glycerol accumulation and results in dehydration. Generally, it is regarded as a stress adaptive pathway but has an important role in the establishment of infection into host cells (21).

3.1.2. Cek1 (*C. albicans* ERK-like Kinase1) mediated MAPK pathway

Cek1p MAPK pathway is activated in response to the nutritional starvation and mediates vegetative growth, helps in the hyphal development and cell wall biogenesis. The other significant function of this pathway is the interaction with the host cells and the establishment of fungal infection (22). *CEK1* gene encodes the MAPK homolog known as Cek1p protein. This Cek1 pathway is stimulated by Msb2p (which is the head sensor protein of the Cek1 MAPK pathway). Msb2p belongs to a class of glycoproteins known as mucins which have one transmembrane domain, an extracellular glycosylated domain which contains Ser/Thr/Pro-rich mucin domain alongwith a cytoplasmic domain that regulates cytosolic signaling molecules (23). A second sensor of the Cek1 pathway is the Sho1p required for Cek1 activation which further transmits the signal via Cdc42 GTPase to Cek1 (24). On the other hand, Cpp1p removes the phosphate group

Table 1. Various kinases along and their functions in *C. albicans*

Protein Kinases	Class	Function	References
Ypd1p, Sln1p, Ssk1p, and Ssk2p/Ssk22p	HOG pathway	Low water activity, stress responses	17
Cek1p	CEK pathway	Nutritional starvation and mediates vegetative growth	22
CaCla4p	CEK pathway	Polarized hyphal growth	27
Protein kinase A	cyclic- AMP-dependent protein kinase pathway	stress resistance, cell growth and intermediary metabolism	29
CDKs,Cak1p	Cyclin-dependent kinase pathway	Degrades the cyclins and inhibitors	35, 37
Tor1 and Tor2	Target of rapamycin (TOR) signaling pathway	Cellular growth in nutritional changes	39
CaRas1	Ras mediated signaling pathway	hyphal morphogenesis	43
CaSln1p, CaNik1p/Cos1p and Chk1p	Two component histidine kinases	hyphal development, morphogenesis, osmoadaptation, cell wall regulation and virulence	45
Crz1p, Rim101 and Nrg1	Calcium Calcineurin Signaling (CCS) pathway	growth and hyphae elongation, cation homeostasis, cell wall synthesis and regulation of cell cycle	46, 50
AKT (protein kinase B)	Phosphoinositide-dependent protein kinase-1 (PDK1) pathway	Biofilm formation	51, 52

from Cek1 for its negative regulation (25). A study by Puri *et al* (26), demonstrated that (Sap8) Sap-mediated proteolytic cleavage of Msb2p is essentially required for activation of the Cek1 MAPK pathway in response to environmental changes. In *C. albicans*, two pathways which involve transcription factors Ste12p (Cph1p) and Phd1p (Efg1p) are functional together but independently also promotes filament development whereas for the formation of polarized hyphal growth, CaCla4p kinase is involved. *CEK* gene activates and functions in serum-induced mycelial colonies formation and induces virulence to candidal cells (27). Cek2 is also involved in the mating but has no role in modulation of antifungal activity (28).

3.2. cyclic- AMP (adenosine monophosphate) -dependent protein kinase pathway

cAMP-dependent protein kinase or protein kinase A (PKA) pathway regulates vital processes in cells. This pathway involves cyclic AMP which acts as a key player produced by ATP. There is role of adenylate cyclase and phosphodiesterase enzymes. The dual regulation of this pathway occurs by adenylate cyclase membrane-bound Ras protein and by glucose via a G-protein-coupled receptor system (29). This protein consists of two catalytic units and two regulatory units. The pathway starts with the binding of cAMP to the regulatory subunit of PKA, in turn liberating the catalytic subunit, which in turn phosphorylates the target proteins involved in stress resistance, cell growth and intermediary metabolism (30). Intracellular cAMP is produced by enzyme CaCdc35p in *C. albicans*. The catalytic unit of PKA are Tpk1 and Tpk2 and transcription factors Efg1 and Flo8 are found in *C. albicans* (31, 32).

3.3. Cyclin-dependent kinase pathway

Cyclin dependent kinase pathway is involved in the regulation of cell cycle. Cyclin-dependent kinases (CDK) are key players in this pathway and systematically coordinate the cell cycle through intracellular and extracellular signals. CDK modulate the activity by associating with regulatory subunits like inhibitors, cyclins and assembly factors by various mechanisms like reversible protein phosphorylation, transcriptional regulation, subcellular localization and by selective proteolysis (33, 34). Cyclins are important for the cyclin kinase activity as they bind to cyclin kinases and leads to phosphorylation. CDKsare regulated at the transcriptional level and synthesized or degraded periodically during the cell cycle and degraded by

ubiquitin-mediated proteolysis. In *S. cerevisiae*, only single Cdc28 unit is found to regulate the cell cycle cascade (35). The regulation of Cdc28 requires the action catalyzed by the CDK-activating kinase gene *CAK1* involved in all phases of the cell cycle to degrade the cyclins and inhibitors (36). Cak1p belongs to the serine/threonine protein kinases family. Far1p and Sic1p are two well known Cdc28p inhibitors. Far1p is required for the arrest of pheromone-induced cell cycle and inhibits the kinase activities of Cln1p-Cdc28p. Sic1p acts as a specific inhibitor of cyclin-Cdc28p complexes. It also inhibits premature S-phase entry in G1 phase before bud initiation and duplication of spindle pole body (SPD). Along with that it also inhibits the Clb-Cdc28p activity which promotes mitotic exit (37).

3.4. Target of rapamycin (TOR) signaling pathway

TOR related protein kinases have been identified in *S. cerevisiae* as a target of the antifungal and immunosuppressive agent rapamycin. TOR is a part of the phosphoinositide 3-kinase-related protein kinase family, which was originally reported from yeast, *S. cerevisiae* (38). TOR signaling pathway, plays an important role in various crucial growth-related phenomenon of yeast. TOR complex 1 (TORC1) and TOR complex 2 (TORC2), are the two functionally distinct TOR complexes, which has been also reported in *Schizosaccharomyces. pombe, C. albicans and Cryptococcus neoformans* and mammals (39). Two Tor proteins known as Tor1 and Tor2 have been recognized that are involved in regulating cellular growth in response to nutritional changes. In *S. cerevisiae*, it is found that Tor signaling by FKBP12-rapamycin stimulates autophagy, induces expression of retrograde response (RTG), inhibits translation and represses ribosomal gene expression (40). Contrary, in *C. albicans*, only one homolog Tor1 protein is present responsible for responses to nutrient starvation.

3.5. Ras-mediated signaling pathway

Ras proteins are small G-binding proteins. Ras activation depends on the exchange of bound GDP for GTP and its deactivation through hydrolysis of GTP to GDP (41). Ras signaling enhances GPI-GnT activity. The activation of Ras signaling pathway is initiated by a conformational change that occurs when it binds to GTP. The inactivation occurs when it is GDP-bound conformation through the exchange of GDP into GTP via Ras-GTPase-activating factors (Ras-GAPs) and guanine nucleotide exchange factors (Ras-GEFs) (42). Of the two Ras proteins, in *C. albicans* only CaRas1 (a close homolog of *S. cerevisiae* Ras2) enhances GPI-GnT activity. It has been studied that it is the key player in activation of Ras signaling as well as hyphal morphogenesis (43).

3.6. Two component histidine kinases

They are two-component system based signaling pathway. It is composed of membrane-associated sensor protein (histidine kinase, HK) and a cytoplasmic, response regulator (RR) protein which typically acts as a transcription factor to adapt cells to the environmental signal (44). In *C. albicans*, there are three two-component histidine kinases called as CaSln1p, CaNik1p/Cos1p and Chk1p along with three response regulators know as *SRR1* (Stress response regulator 1), *SSK1* (Suppressor of sensor kinase) and *SKN7* (Suppressor of Kre Null). They work as sensor proteins that can mediate signaling which in turn regulates hyphal development, morphogenesis, osmoadaptation, cell wall regulation and virulence (45).

3.7. Calcium Calcineurin Signaling (CCS) pathway

The CCS pathway is the main calcium-signaling pathway that is involved in cell survival under various environmental variations. It mediates influx of calcium ions via Cch1-Mid1 (found in fungal cell membranes) channel for the activation of calcineurin and Crz1p (downstream transcription factor). Calcineurin or protein phosphatase 3 is a calcium and calmodulin dependent serine/threonine protein phosphatase. Calcineurin is a protein, highly associated with growth and hyphal elongation, cation homeostasis, cell wall synthesis and regulation of cell cycle (46). The study on CCS signaling has demonstrated that Crz1p is a major mediator of calcineurin-activated gene expression. (47). It has been also reported that phosphorylated form of Crz1p accumulates in the cytosol. When there is an

increase in Ca^{2+} ions, the activated calcineurin dephosphorylate Crz1p, which leads to its nuclear localization (48). There is a presence of C2H2 zinc fingers motif in Crz1p that combines to a specific element present on the promoter in the genes called CDRE (calcineurin-dependent response element) that are required for Ca2+ and calcineurin-dependent gene expression (49). Calcineurin, Crz1p, along with Rim101 and Nrg1 (having a similar role to Crz1) are involved in the pathogenicity of Candida hence devising new drug targets (50).

3.8. Phosphoinositide-dependent protein kinase-1 (PDK1) pathway

It is a Phosphoinositide-dependent protein kinase-1 (PDK1) signaling pathway in which PDK1 is an important protein, which mediates PI3K-AKT pathway, making it a potential drug target (51). The structure of PDPK1 can be divided into two domains; the kinase or catalytic domain and the PH domain. The interaction of PDPK1 with phosphatidylinositol (3,4)-bisphosphate and phosphatidylinositol (3,4,5)-trisphosphate is accomplished by PH domain. This interaction is important for localization and activation of some of membrane associated PDPK1's substrates including AKT (protein kinase B) (52). The activity of PDK1 is mainly regulated by allosteric and orthosteric modulators, by binding to the PIF (Interacting Fragment) pocket of the PDK1. Perhaps, there are number of advantages of binding of allosteric modulators to the less conserved PIF-pocket, like high specificity, minimum side effect and reduced toxicity (53). However, the binding of the allosteric inhibitors to the PIF-pocket is shown to cause the rearrangement of αB and αC helices in the inert conformation, which leads to the inhibition of PDK1 activity (54).

4. ROLE OF KINASES IN *C. ALBICANS*

4.1. In Yeast to hyphal development/Morphogenetic switching

C. albicans being diploid is capable of undergoing morphogenetic transition from yeast to filamentous form under nutrient starvation conditions. There are three interchangeable morphological forms critically important for pathogenesis, known as yeast form, pseudohyphae and true hypha (55)**.** The hyphal form is induced under certain conditions like nitrogen starvation, high temperature, neutral pH etc. (56). The hyphal formation is a multifactorial phenomenon governed by more than one signaling pathway comprising protein kinases (57). Dimorphism is crucial in serving many functions like adhesion to host cell, escape from phagocytic cells and invasion into another host cell (58). The hyphal development is activated by MAPK kinase pathway or cAMP protein kinase A pathway. Transcription factor, Cphlp which is homologue of Stel2p in *S. cerevisiae* is involved in the hyphal formation along with other transcription factors like Cst2Op, Hst7p (59). Under starving conditions, there is an inactivation of *CPP1* which induces hyphal development. Cpp1p protein suppresses the hyphal development at ambient temperature. Another transcription factor, Efg1p is activated in cAMP protein kinase A cascade (60). The *CAP1* gene mediates induction of yeast to hyphal transition and favors filamentous growth to give a response to serum or other environmental conditions. Ssk1p and Chk1p are essential for the yeast to hyphal transition (13).

HOG pathway represses the serum-induced yeast-to-hypha transition in *C. albicans* suggesting its role as another determinant of virulence. In *C. albicans*, *TUP1* gene is responsible for hyphal development (61). It has been reported that repression of hyphal gene was dependent on Ssn6-Tup1 complex which in turn depends on HOG1-dependent expression or activation of a DNA binding protein (62). Mds3 which is a negative regulator of Tor signaling pathway involved in the yeast-hyphal transition. *RAS2* gene acts upstream of signaling pathways which can lead to hyphal differentiation whereas *Candida RAS1* gene is essential for hyphal differentiation in response to serum (63).

Cyclin dependent kinases are also involved in cytoskeleton polarization for morphogenesis in candidal cells. Cdk cdc28 binds temporarily to cyclins namely Ccn1 and Hgc1. The Cdc28-Ccn1 pair binds to septin complexes which in turn results in phosphorylation of Cdc11 which is a septin

cytoskeleton protein required for hyphal development (36). Hsl1p-Swe1p-Cdc28p is essential for cell elongation in morphogenesis and imparts virulence to candidal cells. Cdc28 exerts inhibitory phosphorylation on Tyr-19 via Swe1p. It is also involved in the delay of the cell cycle in G2 phase and it is a 'morphogenesis checkpoint pathway' (64). Swe1p is also regulated negatively by Hsl1p protein kinase. Under low Cdc28p activity, Swe1p activates and leads to G2 delay which results in elongated bud formation. Relatively, Hsl1p activates when colocalized with the cytoskeletal proteins septins, around the neck. Cyclin namely Cdc11p or Cdc12p, a component of the septin complex, interacts directly with Hsl1p. It acts as a reliever of the autoinhibition which is imposed by the kinase inhibitory domain. Hsl1p localization results in inhibition of Swe1p which allows the cells to undergo mitosis (65). Similarly, the calcium signaling of a cell is required for various physiological activities in yeast, which includes morphogenesis, and virulence (66,67).

4.2. Cell to cell adhesion and biofilm formation

Tor protein kinase is involved in the cell to cell adhesion in *C. albicans*. It regulates transcriptional regulation of cell surface adhesins and results in cell adherence. The inhibition of Tor1 facilitates the increase in expression of genes encoding the adhesins Als1p, Als3p, Hwp1p and Ece1p by genes *ALS1, ALS3, HWP1,* and *ECE1* respectively which in turn promotes biofilm formation (68, 69). Thus, in other words, it can be said that Tor1 inhibits cell adhesion by repression of adhesin genes expression. However, Tor1 regulates and governs cell adhesion by multiple mechanisms. It controls the expression of *NRG1* and *TUP1* by downregulating Efg1 and Bcr1 (69). Mds3 is involved in a variety of morphogenetic processes, including the yeast-hyphal transition, chlamydospore formation and biofilm formation (70). A study by Flanagan *et al* (71) has shown that TOR-Activating GTPases Gtr1 and Rhb1 regulate the biofilm formation and nutrient starvation. Ras1-Cyr1-PKA signaling is found to be important as it regulates the expression of adhesins, such as Als1p, and also control the activity of the key transcription factors *EFG1*, *TEC1* and *BCR* (72). The calcium signaling is also required for adhesion, drug tolerance in *C. albicans* (66, 67). The PDK1 orthologs (Pkh kinases) of PDK pathway is also involved in the biofilm formation (73).

4.3. Oxidative stress response

Under high oxidative potential, there is a formation of reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^\cdot$) which damages cellular components like DNA, lipids, and proteins (74). All these factors have toxic effect on yeast cell which leads to cell death. To cope up with oxidative stress, yeast cells have developed several antioxidant mechanisms (75). The activation of detoxifying enzymes such as superoxide dismutases and catalases, to prevent the ROS action, nonenzymatic scavenging substances are formed (76). In *S. cerevisiae*, Yap1 transcription factor is involved in the oxidative stress response. , A Yap1 homolog called as Cap1 in *C. albicans* is involved in mediating the oxidative stress resistance. HOG pathway plays an important role in an acute and adaptive response to oxidants (77). A study by Alonso-Monge *et al* (21)demonstrates that Hog 1 MAP kinase gene plays a crucial role in the oxidative stress response via different mechanisms from *CAP1*. The activation of Hog1 occurs due to phosphorylation in the presence of H_2O_2 or by increase in external osmolarity which depicts the specificity of the Hog1-mediated response to oxidative stress. This study broadens the role of MAP kinase in morphogenesis and osmoadaptation (21).

4.4. Chlamydospore formation

Chlamydospores are thick-walled cells which are formed under certain environmental conditions like defined nutritional media, low temperature and oxidative stress. Its formation is the distinctive morphological feature of *C. albicans (*78). They usually arise as elongated suspensor cells which are situated on pseudohyphae or hyphae. It has been reported that six genes namely *ISW2, MDS3, RIM13, RIM101, SCH9,* and *SUV3* are required for efficient chlamydospore formation. The

mutations in *ISW2*, *SCH9* and *SUV3* completely remove chlamydospore formation whereas mutations in *RIM13*, *RIM101*, and *MDS3* delays normal chlamydospore formation (79). Efg1p is the protein which belongs to a group of bHLH proteins termed as APSES-proteins. Efg1p as a key regulator of *C. albicans* morphogenesiswhich influences yeast hyphae interconversion as well as chlamydospore formation (80). A study by Alonso-Monge *et al* (21) stated that *HOG1* gene also takes part in chlamydospore formation.

4.5. Cell wall remodeling and masking of major cellular components

The candidal cell wall is composed of a dynamic structure comprising of β-1,3-glucans which is covalently linked to β-1,6-glucans and chitin, along with matrix composed mainly of mannose-glycosylated proteins (81). It was reported that Cek1 plays a role in the maintenance and structure of candial cell wall by regulating the β-glucan exposure. It also maintains the cell wall glycoproteins by regulating the mannosylation status. Cek1 pathway was also found to be responsive against the antifungal agents like caspofungin and tunicamycin by masking the cell surface damage (25). Similarly, Cek1 pathway is also responsive to the glycosylation defects in the cell wall (82). Two component histidine related gene *CHK1* is involved in the regulation of cell wall mannan and glucan biosynthesis (45). The activation of HOG1 pathway also triggers cell wall modification. A study by Alonso-Monge *et al* (21) suggested the role of *PBS2* gene in maintaining cytoskeleton by β- (1,6)-glucan assembly. The activation of glycosylphosphatidylinositol anchor biosynthesis through the GPI-N-acetylglucosaminyl transferase (GPI-GnT) is accomplished by Ras signaling. (44).

Cell wall surface plays a crucial interface between host and pathogen. When the microbial cells are phagocytosed by host cells, they have highly conserved pathogen-associated molecular patterns, which are recognized by different pathogen recognition receptors (PRRs) which include C-type lectins and Toll-like receptors (TLRs) (83). The recognition of these PRRs further mediates microbial killing as well as antigen presentation which finally results in the production of proinflammatory cytokines. In *C. albicans,* the β- (1,3)-glucan on cell wall is recognized by C-type lectin receptor called as Dectin 1 which is expressed by myeloid cells (84). The coating of mannoprotein on cell surface helps the candidal cells to escape from the host cell recognition. The Cek1 MAPK kinase pathway favors the β- (1,3)-glucan masking and alters the binding of Dectin1 to the β-glucan which helps in evasion of immune response and recognition by host cell . It has been reported that Cek1p MAPK kinase cascade involves the Ste11-Hst7-Cek1 which aids in controlling the β (1,3)-glucan masking in *C. albicans* (85). Another study also demonstrated that exposure of α-1,2 and β-1,2-mannosides in the cell wall was mediated by Cek1-mediated MAP kinase pathway which modulates immune recognition. Additionally, even the calcium signaling is also required for maintaining cell wall integrity (66, 67).

4.6. Mating or Pheromone pathway

Mating in yeast cells is simple unlike complex eukaryotes and sexes are designated as a and α (86). They communicate by signaling molecules called pheromones. The pheromones are detected by receptors on cell surfaces and communication is further disseminated and preceded by heterotrimeric G protein complex that activates mitogen-activated protein (MAP) kinase cascade module (87). In *S. cerevisiae*, two mating MAP kinases are involved called as Fus3 and Kss1. Contrary, in *C. albicans*, Cek1 and Cek2 MAP kinases are involved in the mating process. Mating in *C. albicans* relies on a reversible phenotypic switching between two states termed as ‘white’ and ‘opaque’. Wor1 transcription factor is the key player in regulating the white-opaque switch. Ras1-Cyr1-PKA pathway is known to have a role in the white-opaque switch (88).

4.7. Cell cycle regulation

Regulation of cell cycle is critical for the precise and appropriate manner as to ensure the sequential coordinated events to bring equal distribution of genetic material to daughter cells. In higher eukaryotes, the cell cycle is controlled by

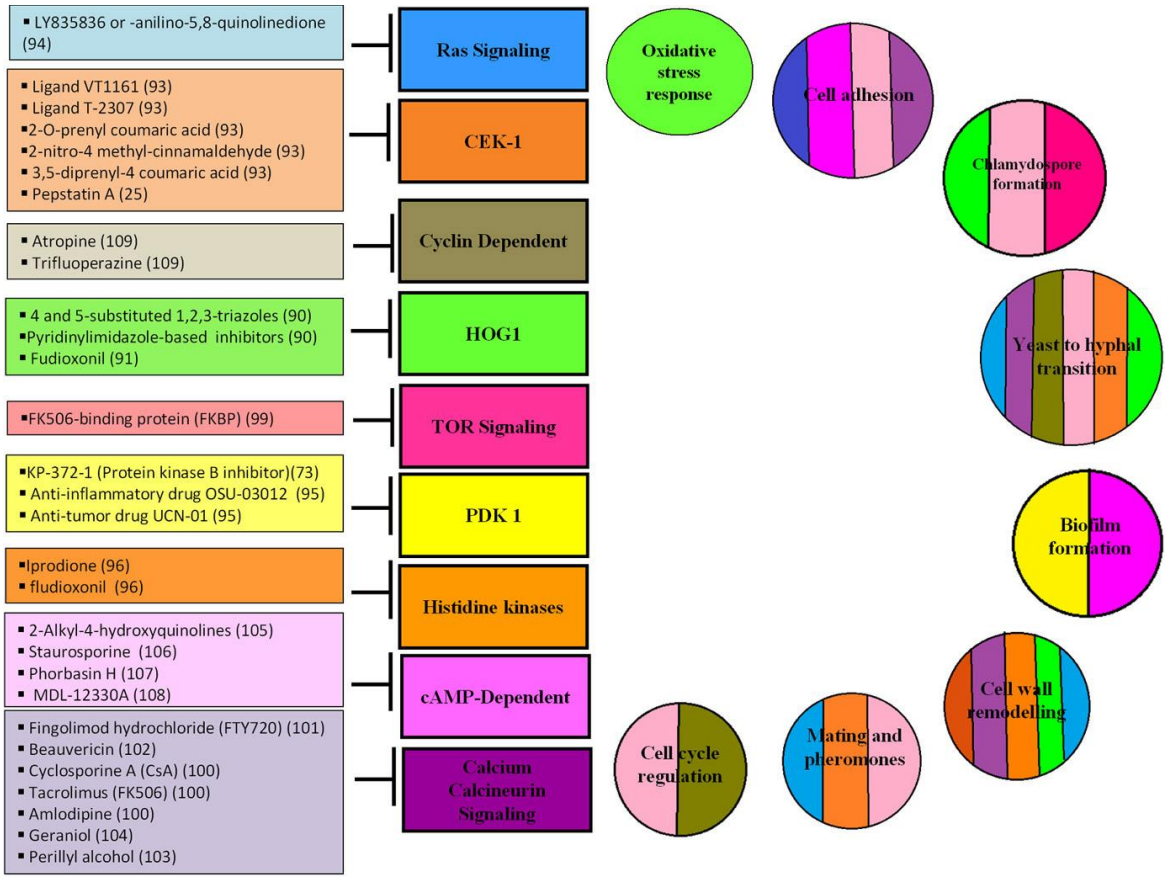

Figure 1. Various inhibitors designed to target protein kinase pathways governing several functions in *C. albicans*. The color codes depict common functions shared by one or more pathways derived from previous literature.

activation or deactivation of cyclin dependent kinases (CDKs) (89)

5. INHIBITORS OF KINASES IN *C. ALBICANS*

5.1. Inhibitors of HOG pathway

HOG pathway is considered to be a potential intracellular drug target with reduced toxicity to the host, due to the fact that, TCS is conserved in a broad number of living organisms, from microorganisms to plants, except mammals. The compounds, 4- and 5-substituted 1,2,3-triazoles, and pyridinylimidazole-based inhibitors, potentially have been demonstrated to specifically inhibit Hog1 in *S. cerevisiae* (90). Simultaneously, HOG pathway overactivation can also be lethal to a pathogen, as observed by the activity of fludioxonil (Figure 1). The study suggests that development of drugs which targets the negative regulators of the HOG pathway could provide better results in killing a pathogen (91).

5.2. Inhibitors of CPH1 (*Candida* PseudoHyphal regulator) -MAPK (Mitogen Activated Protein Kinase)

The inhibition of *CPH1* could prove as a beneficial drug target for *Candida* therapy, because of the abundance of functions contributing to its virulence. The interaction of ligand and receptor has a leading role in all cellular metabolic activities (92). The in silico work conducted by Jha *et al* (93), demonstrated that several ligands, like, VT1161, T-2307, 2-O-prenyl

coumaric acid, 2-nitro-4 methyl-cinnamaldehyde, and 3,5-diprenyl-4 coumaric acid, profoundly blocks Cst20, Hst7, and Cek1. Such ligands can disrupt the cell wall organization, block biofilm formation and topography, due to the ability of the lead molecule to interact with the kinase by binding to the amino acid residues (93). Pepstatin A which is the known inhibitor of aspartic proteases inhibits the Msb2 shedding from the cell surface and phosphorylation of Cek1 (25).

5.3. Inhibitors of Guanylate cyclase

One of the kinases, reported to be essential for the survival of *Candida* is CaMps1p, i.e., *C. albicans* monopolar spindle 1, which also shares some sequence homology with hMps1p, a human ortholog. A study conducted by Tsuda (94), demonstrated the recombination of a catalytic domain of CaMps1p labeled as LY83583, which is 6-anilino-5,8-quinolinedione. LY83583, also termed as a guanylate cyclase inhibitor, inhibited the activity of CaMps1p kinase, hence reducing the growth of *Candida* cells. Additionally, LY83583 was found to have inhibition which is specific for CaMps1p without affecting hMps1p activity, suggesting that LY83583 could be an ideal inhibitor for anticandidal therapy (94).

5.4. Inhibitors of Phosphoinositide-dependent protein kinase-1 (PDK1)

One of the PDK1 inhibitors is KP-372-1, a potential molecule that inhibits the PDK1 orthologs (Pkh kinases) is involved in the biofilm (73). Two other PDK1 inhibitors, OSU-03012 and UCN-01, are also known for their activity against biofilms of *C. albicans* (95). Therefore, the search for inhibitors of PIF-pocket of PDK1, could provide an ideal target for drug development.

5.5. Inhibitors of Histidine kinase pathway

It has been reported that two fungicides called as Iprodione and fludioxonil targets the histidine kinases by inhibiting *CaN1K1* and *COS1* transduction system governing osmotic transduction system which disturbs glycerol synthesis and inhibits the hyphal formation (96).

5.6. Inhibitors of Target of rapamycin (TOR) signaling pathway

Rapamycin, a microbial product, specifically inhibits TORC1, while TORC2 is indirectly sensitive to rapamycin, and inhibits, *C. albicans* and *C. neoformans* (97, 98). Rapamycin forms a complex with a cytoplasmic protein, i.e., FK506-binding protein (FKBP) receptor and inhibits the TORC1 activity (99). The Mds3, Sit4, Tor1, and Tco89, members of the TOR pathway have crucial roles in morphogenesis, which in turn demonstrates their role in virulence of *C. albicans* (98, 100,). Further studies on Mds3, Sit4, Tor1, and Tco89 could help in developing a structural modification of rapamycin to treat *C. albicans* infections.

5.7. Inhibitors of calcium cell survival (CCS)

A modulator, sphingosine 1-phosphate (S1P) receptor, known as fingolimod hydrochloride (FTY720), acts on calcium signaling in *Candida*. It has a potential to suppress the growth of *Candida* by inducing a dose-dependent calcium increase, due to an influx of the molecule across the cytoplasmic membrane (101). Beauvericin is reported to enhance cytoplasmic calcium which plays an important role in the apoptosis, particularly Ca^{2+} overload of mitochondria (102). Cyclosporine A (CsA), tacrolimus (FK506) and amlodipine in combination with fluconazole, shows excellent synergistic effect against resistant *C. albicans*. Cyclosporine is known inhibitor of calcineurin and down regulates expression of *ALS3*, *HWP1*, *CDR1*, *MDR*, *ERG11* genes (100, 50). Even natural compounds belonging to class of phenolic compounds, terpenoids, alkaloids were found to inhibit the calcineurin signaling pathway. A phenolic compound like perilyll alcohol and terpenoid like geraniol have been reported to disrupt the calcineurin signaling (103, 104).Therefore, specific inhibitors for the calcium signaling pathway which is involved in cell survival could provide a novel platform for the development of antifungal agents.

5.8. Inhibitors of cAMP pathway

Various compounds have been studied as inhibitors for targeting cAMP pathway namely 2-Alkyl-4-hydroxyquinolines which is derived from marine *Streptomyces* sp. inhibiting candidal

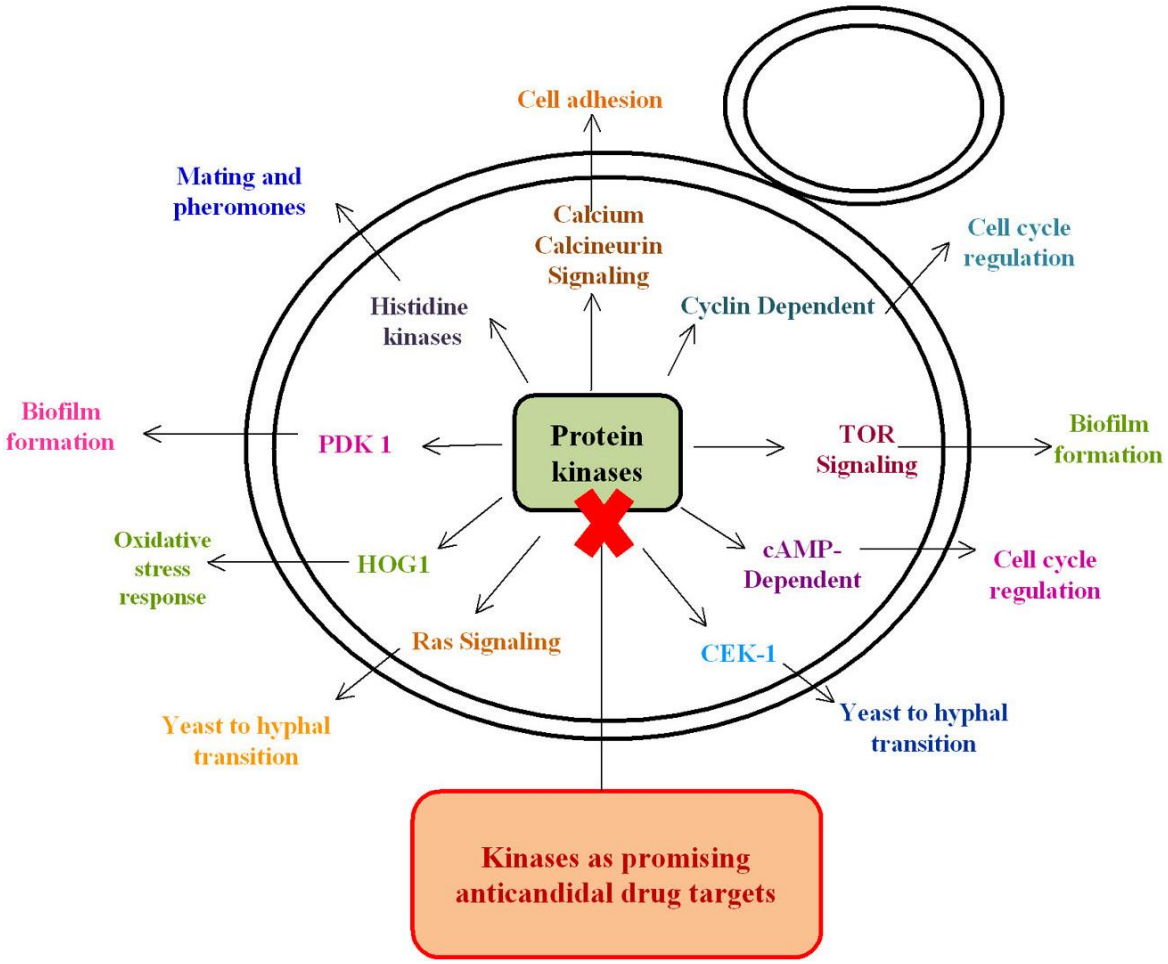

Figure 2. Model depicting protein kinases as potential anticandidal drug targets.

hyphal development (105). Another compound staurosporine targets cyclic AMP (cAMP)-dependent protein kinase A and adenylyl cyclase Cyr1 resulting in abrogated fungal drug resistance and morphogenesis (106). Phorbasin H which has been isolated from marine sponge Phorbas sp inhibits the yeast-to-hyphal transition in *C. albicans* (107). Mammalian adenylate cyclase inhibitor MDL-12330A has been tested and found to inhibit the cyclic AMP signaling pathway in *C. albicans* (108).

5.9. Inhibitors of Cyclin dependent pathway

The inhibition of cell cycle was demonstrated by delaying the expression of cyclins. The compounds named as atropine and trifluoperazine were found to prolong the cell cycle by delaying the expression of G1 cyclins (109).

6. CONCLUSION

Phosphorylation events regulated by several kinases are strong circuits governing fundamental physiology and pathogenicity of fungi such as *C. albicans*. Protein kinases, or also called as phosphotransferases can phosphorylate the target proteins by simply tranfering the phosphate groups and forming a covalent bond to the side chains of amino acids like serine, threonine or tyrosine. Thus targeting these kinases may result in corresponding inhibition of multiple cellular

processes that are crucial for the viability of the fungi (Figure 2). Modulation of kinase activity could be another therapeutic approach for the treatment of candidal infections. Elucidating the crystal structures of the kinase domain could further aid in designing molecules targeting these kinases that would lead to the generation of broad-spectrum antibiotics to be exploited in future therapeutics. The designing of low molecular weight compounds which can easily mimic the protein kinases may be a better way to target the protein kinases.

7. FUTURE PROSPECTS

Protein kinases serve as the principal regulator of the biological cycle, so any disturbances in the interaction of protein kinases in the cell cycle regulation could disrupt the pathogenicity of pathogenic fungi. Protein kinases are subject of exclusive research as they are the key players in the signaling pathways. Future prospects of understanding the protein kinases could open the new gates and opportunities for scientists and chemists working in the field of drug development. The computational studies could help in elucidation of the structures and designing of inhibitors for targeting protein kinases.

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Abbreviations: MAP: Mitogen Activated Protein, HOG: High Osmolarity Glycerol, Cek1: *C. albicans* ERK-like Kinase1, cAMP: Cyclic-Adenosine Monophosphate, TOR: Target of Rapamycin, CCS: Calcium Calcineurin Signaling, PDK1: Phosphoinositide-dependent Kinase

Key Words: MAP Kinase pathways, HOG pathway, Target of rapamycin, TOR, Signaling pathway, Phosphoinositide-dependent protein kinase-1, PDK1, Cek1, Candida albicans, ERK-like Kinase1, MAPK, Review

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