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Molecular Drug Targets in *Candida glabrata*

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Abstract
Incidence of candidiasis has increased in past decade. Epidemiology is reported shifting from *albicans* to non-*albicans Candida* (NAC) species like *C. glabrata*, which is intrinsically resistant to azole drugs. No new antifungal has come into practice from last decade. Rising resistance to existing antifungal (in clinical isolates of *Candida*) have necessitated the need for new antifungals. New molecular drug targets need to be explored for the development of novel antifungal drugs. The drug targets are oftenly the cellular proteins of various metabolic pathways (like ergosterol synthesis-, cell wall biogenesis-, calcium-calcineurin- and DNA checkpoint pathways etc.), having no significant similarity with host proteins to overrule the possibility of side effects. In this review, some potential proteins of *C. glabrata* and their pathways are discussed in context to explore their potential as drug target for antifungal drug development.

Keywords- *Candida glabrata*, Molecular Target, Drug Target, Kre1, Kre2, Cch1, Mid1, Cdr1, Rox1, Upc2B.

1. Background

Candida is an opportunistic and commensal fungal pathogen mainly colonizing human mucosal surfaces. The morbidity and mortality associated with *Candida* infection is increasing at an alarming rate worldwide, as it is primarily responsible for hospital acquired infections (Nosocomial infections) and blood stream infections (Giri and Kindo, 2012). About 90% of all *Candida* infections are caused by five major species namely, *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis* and *C. krusie* (Guinea, 2014). The epidemiology of *Candida* infection has been changed in last two decades and varies from place to place. The trend of *Candida* caused infection is now shifting from *albicans* to non-*albicans Candida* species worldwide (Guinea, 2014; Yapar, 2014). *C. glabrata* is major cause of *Candida* infection in Northern Europe and U.S.A., while *C. parapsilosis* is reported in most cases in Spain and Brazil *C. glabrata* stands at second position after *C. albicans*, for causing diverse infections, ranging from superficial mucosal to disseminated (Pfallar and Diekema, 2010). There are numerous reports on the *Candida* caused infections from all around the world. In Paraguay, the rate of *Candida* associated catheter related bloodstream infection (CRBSI) in ICU patients has been found to be around 2.5% of total ICU admitted patients during 2009-2012 (Cueller et al., 2008). According to an eight-year surveillance report of *Candida*, Blood Stream Infections (BSI) in Saudi Arabia, 34.1% cases of *Candida albicans* and 65.9% of non-*albicans Candida* species have been reported where the approximate 12-month mortality

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rate was 50% for *C. albicans* and 57.8% for non-*albicans Candida* species (Al Thaqafi et al., 2014).

In India, the distribution of *Candida* species also varies from region to region but *C. tropicalis* is the most prevalent in the clinical samples followed by *C. parapsilosis, C. albicans, C. glabrata, C. rugosa, C. hemulonii, C. guilliermondii* (Kaur et al., 2014; Tak et al., 2014). Limited numbers of drugs are available against *Candida* due to several factors which favors the virulence of pathogen like dimorphic transition, permeability, resistance to phagocytosis and ability to grow well at 37^{o}C (Nobile and Johnson, 2015). Increasing reports on *Candida* resistance and increasing cases of invasive candidosis/candidiasis have led the attention of researchers to develop effective therapeutics.

Identification of drug targets is the foremost step for designing a drug. A good target must be crucial for survival and virulence of pathogen and must not share similarity with any of the host proteins (Spitzer et al., 2011; Pierce and Lopez-Ribot, 2013). Approach of reverse genetics (gene deletion and gene silencing) is useful in the identification of new drug targets. (Lindsay, 2003; Sturgeon et al., 2006).

Several genes have been identified in *Candida* for viability and pathogenesis, which belong to transcription factors, adhesins, drug transporters, membrane channels and kinases etc. (Roemer et al., 2003; Sangamwar et al., 2008; Thompson et al., 2011). Some of the important pathways of *C. glabrata* and their effector proteins are discussed in this review as putative drug targets.

2. Molecular Drug Targets

C. glabrata is associated with high mortality and morbidity and is also resistant to antifungals. The problem of candidiasis could be addressed by identification of new molecular drug targets for development of novel antifungal. Generally, the members of essential metabolic pathways and others which are crucial for survival and growth of pathogen, could serve as a good drug target. Few pathways whose members could be a good target are cell wall biogenesis, calcineurin pathway, voltage gated Ca^{2+} channels, transcription factors, ergosterol synthesis, DNA checkpoint pathway (Calendrone and Cihlar, 2002; Gozalbo et al., 2004; Ton and Rao, 2004; Belardetti and Zamponi, 2012; Liu et al., 2015).

3. Calcium-Calcineurin Pathway

The essential components of Ca^{2+} signaling machinery are conserved and fundamentally similar in eukaryotes. The components of Ca^{2+} signaling and homeostasis pathway are Ca^{2+} channels and transporters, Ca^{2+} sensors, and signal transducers (Ton and Rao, 2004). Intracellular Ca^{2+} concentration increases in response to external stresses like thermal stress, oxidative stress, hyper-osmotic stress, cold stress, alkaline stress and ethanol stress through

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increased Ca^{2+} uptake and also by secretion of Ca^{2+} from internal compartments of cell (Liu et al., 2015). Also, in response to Endoplasmic Reticulum (ER) stress, exposure to mating pheromones, and depletion of Ca^{2+} inside ER, Ca^{2+} influx system (HACS & LACS) in plasma membrane gets activated and Ca^{2+}enters into cytosol via Cch1/Mid1 complex. This leads to increased intracellular Ca^{2+} concentration (Martin et al., 2011). This increased Ca^{2+} concentration affect various cellular processes like cell cycle progression, hyphal branching, gene expression etc., and also modulates signaling cascade and activates calcineurin pathway (Kraus et al., 2005).

The components of calcium-calcineurin pathway are: -

- Ca^{2+} influx system on plasma membrane - consist of HACS and LACS (Liu et al., 2015).
- Calmodulin (CaM) - Ca^{2+} sensor, binds maximum up to three Ca^{2+} ions (Luan et al., 1987).
- Calcineurin (CN) - a highly conserved serine/threonine protein phosphatase, consist of two subunits, one is catalytic subunit A (CnA; encoded by CNA1 and CNA2) and another one is regulatory subunit B (CnB; encoded by CNB2). CN depends on Ca^{2+}/calmodulin for its activation (Liu et al., 2015).
- *CRZ1/TCN1* & *PRZ1* – C_2H_2-type zinc finger transcription factor gets translocated in nucleus from cytoplasm when dephosphorylated (Liu et al., 2015).

Under stress conditions, the intracellular Ca^{2+} concentration increases and is sensed by CaM. Three Ca^{2+} ions bind to the EF-hands motif of CaM. This Ca^{2+}/CaM binds to CnA and promotes the binding of Ca^{2+} to high affinity Ca^{2+} binding sites on CnB. This attachment leads to the activation of protein phosphatase, CN which in turn dephosphorylates its down-stream transcription factor, *CRZ1/TCN1* & *PRZ1*, and translocate it from cytoplasm to nucleus. Here, it initiates the expression of Ca^{2+} - ATPases genes namely *PMR1*, *PMR2* and *PMC1* and glucan synthase gene *FKS2*, by binding to CN-dependent responsive element. Subsequently, the Ca^{2+} uptake by organelles increases and release of Ca^{2+} from vacuole reduces, resulting in decreased intracellular concentration of Ca^{2+} (Ton and Rao, 2004; Liu et al., 2015).

4. Targets from Ca^{2+} Influx System (Voltage Gated Calcium Channels)

Voltage Gated Calcium Channels (VGCCs) plays a very important role in regulating various physiological function in an organism. They regulate the entry of calcium into electrically excitable cells. Amlodipine besilate, an inhibitor of Ca^{2+} channels have been proven as an effective anti-*Candida* drug (Gupta et al., 2016). Ca^{2+} channels get activated in response to membrane action potential and leads to Ca^{2+} influx into the cell (Catterall, 2011). The presence of Ca^{2+} channels and their role in response to various stress have been described in nearly all fungi (Ton and Rao, 2004). Various cellular processes like pheromone arrest, cell cycle arrest and morphogenesis are controlled by calcium signaling in yeast (Kaur et al.,

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2004). In fungal cell membrane, two different types of Ca^{2+} influx systems are present: one is high affinity Ca^{2+} influx system (HACS) and another is Low Affinity Ca^{2+} influx System (LACS) (Harren and Tudzynski, 2013).

An important subunit of HACS reported in yeast and fungal pathogens is Cch1, homologous to pore forming α_1-subunit of L-type VGCC (Teng et al., 2008). Cch1 consist of four domains and six trans-membrane segments (S1-S6) present in each domain. S4 segment in all domains except IV contain motif of positively charged residues which sense voltage (Martin et al., 2011; Teng et al., 2013). Voltage sensing mediated by S4 segment of Cch1 is poorly conserved in yeast, suggesting response to unusual voltage range (Teng et al., 2013).

Mid1 is *N*-glycosylated integral membrane protein, analogous to regulatory α_2/δ- subunit of mammalian VGCC (Teng et al., 2013). Mid1p have a putative N-terminal signal peptide and several potential trans-membrane α-helices (Iida et al., 1994; Tada et al., 2003). When expressed in mammalian cells, Mid1p functions as a stretch-activated Ca^{2+}-permeable cation channel (Bonilla and Cunningham, 2003; Burchmore et al., 2003). HACS is consisting of a complex of Cch1 and Mid1 (Martin et al., 2011).

Another protein, Ecm7 has been recently reported to be involved in regulation of HACS (Martin et al., 2011). It is a member of PMP-22/EMP/MP20/Claudin super-family and is homologous to λ-subunit of VGCCs (Teng et al., 2013; Liu et al., 2015). *C. albicans* ecm7Δ/Δ mutants under oxidative stress were sensitive and showed attenuated invasion ability in mouse kidney compared to wild-type (Ding et al., 2013). Similarly, *cch1*Δ/Δ or *mid1* Δ/Δ attenuate *C. albicans* resistance to fluconazole and itraconazole, in mouse model (Wang et al., 2012).

Under ER stress, cch1 and mid1 null mutant of *Cryptococcus neoformans* was not able to survive suggesting the requirement of Cch1 and Mid1 in survival of *C. neoformans* under stress and restoration of Ca^{2+} homeostasis (Hong et al., 2010). In neutropenic murine model of invasive pulmonary candidiasis, Δcch1 and Δmid1 *Aspergillus fumigatus* strain showed attenuated virulence (De-Castro et al., 2014). Cch1 gets activated in response to ER stress by mitogen activated (MAP) kinase and its upstream regulator Bck1 (Bonilla and Cunningham, 2003).

The voltage gated calcium channels are well established putative therapeutic targets in wide range of organism and for wide range of diseases as they regulate numerous physiological functions important for survival of cell (Belardetti and Zamponi, 2012). The balance between the intracellular and extra cellular Ca^{2+} level needs to be maintained properly since large increase in cellular Ca^{2+} level might be lethal to cell and leads to Ca^{2+} - mediated cell death. This pathway serves as a very good target for antifungal drug development (Berridge et al., 2003; Ton and Rao, 2004). Amiodarone, a novel antifungal, works on the principle of Ca^{2+} -

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mediated cell death pathway where it stimulates massive influx of Ca^{2+} and leads to cell death via apoptosis (Liang et al., 2011; Mulu et al., 2013). Conservation of Ca^{2+} signaling pathways in many pathogenic fungi including *C. glabrata* suggest their role in cell survival and resistance to compounds that target membrane biosynthesis (Martin et al., 2011). Various calcium channel blockers have also been known that would serve as good antifungal, like Nifedipine, verapamil, diltiazen (Teng et al., 2008; Yu et al., 2013).

While there are some similarities between the components of mammalian and fungal Ca^{2+} signaling pathways but still sufficient structural differences have been reported. Also, due to the essential role of Ca^{2+} signaling pathways in survival of fungal cells, components of this pathway could serve as a potential target for new antifungal drug development (Del Aguila et al., 2003; Liu et al., 2015). A recent study has shown the antifungal properties of amlodipine (an inhibitor of mammalian Ca^{2+} channel), against *C. albicans*, in combination with fluconazole (Liu et al., 2016). Another study has also reported the anti-*Candida* potential of amlodipine besilate (Gupta et al., 2016).

5. Targets from Cell Wall Biosynthesis Pathway

The fungal cell wall is a flexible 3D-structure consists of glucan, chitin, mannan and manno-protein. β-1,3-glucan is the major carbohydrate component of cell wall. It is arranged as triple helical polymer and forms a lattice support structure and provide attachment site for other molecules. Chitin is a simple polysaccharide, homo-polymer of β-1,4-linked *N*-acetyl-glucosamine (GlcNAc) (Lenardon et al., 2010). It is a minor but important component of cell wall and form cross-link complex with β-1,3-glucan. Long chains of chitin form tight anti-parallel structure bonded together by H-bond and are insoluble (Gozalbo et al., 2004; Chaffin, 2008). Chitin and β-1,3-glucan complex constitute inner surface of cell wall (Lipke and Ovalle, 1998). β-1,6-glucan polymer are attached as branch structure to β-1,3-glucan backbone and links components of inner and outer membrane (Lipke and Ovalle, 1998; Lowman et al., 2011). The outer surface consists of extensively *O*- and *N*- glycosylated manno-proteins, formed of mannan, a polymer of mannose (Man) that are covalently linked to proteins (Gozalbo et al., 2004). Cell Wall Proteins (CWP) are of two types:

- GPI – most abundant class of CWPs linked to β-1,6-glucan through GPI.
- Pir – (Proteins with integral repeats) linked to β-1,3-glucan.

Apart from these two classes, CWP have third class of protein that lacks covalent attachment to polysaccharide matrix (Chaffin, 2008). GPI-CWP plays role in maintaining cell shape, hydrophobicity, cell wall biosynthesis and remodeling and limiting permeability (Weig et al., 2004). Few known GPI proteins in *C. Glabrata* are Epa1 (Cormack et al., 1999), Epa 2, 3, 4, 5 (De Las Peñas et al., 2003), Epa 6, 7, a family of three Gas/Phr orthologues (Weig et al., 2001).

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The vital procedure of formation and assembly of cell wall components takes place on plasma membrane. Chitin is synthesized by polymerization of UDP-GlcNAc residues on the plasma membrane surface by chitin synthase in a partially zymogenic state. UDP-GlcNAc residues are synthesized from glucose-6-phosphate in cytoplasm and transported to the plasma membrane for elongation of chitin chain. After the complete synthesis of chitin chain, it gets extruded outside the plasma membrane (Gozalbo et al., 2004).

β(1,3)-glucan synthase is located in plasma membrane where it catalyses formation of β(1,3)-glucan by using its two subunits – a catalytic and a regulatory GTP-binding subunit which takes UDP-Glc from cytosol and attaches it to glucan chain of cell wall. β(1,6)-glucan linear chains form in endoplasmic reticulum and then transported to cell wall where it gets mature and finally assemble with other components of cell wall (Ruiz-Herrea, 1992).

Cell wall is a complex and dynamic outermost structure and plays important role in interactions of micro-organism with environment, including host and therefore also have role in fungal pathogenicity (Sanjuán et al., 1995; Chaffin et al., 1998). Hence, fungal cell wall could be a potential target for new antifungal drug development. The fungal cell wall contains many unique components that may are of great interest for development of better therapeutic, as in case of bacteria, drugs targeting their peptidoglycan are potential drug molecules. The genes involved in **cell wall biogenesis** is potential categories that can be targeted (Gozalbo et al., 2004).

Products of followings genes may be good drug targets from cell wall biogenesis pathway:
5.1 KRE1*:* *KRE1* encodes a secreted, O-glycosylated, threonine- and serine-rich agglutinin-like protein necessary for the addition of β-1,6-linked outer chains to a core of glucan structure; it can be found mainly at the cell wall (Boone et al., 1990; Roemer and Bussey, 1995). The plasma membrane-associated, GPI-anchored Kre1 plays role in extending the chain by adding linear -1, 6-glucan onto a highly branched acceptor glucan (Lesage and Bussey, 2006). *KRE1* disruption leads to 40% reduction in overall β-1, 6-glucan content of cell wall. It appears in late stage of β-1, 6-glucan synthesis and assembly (Breining et al., 2004). It serves as K1 killer toxin membrane receptor (Roemer and Bussey, 1995). Kre1 have two hydrophobic ends where Kre1 is GPI linked through C-terminal domain in plasma membrane (Breining et al., 2004) while N-terminal signal peptide directs kre1 in secretory pathway (Roemer and Bussey, 1995).

5.2 KRE2: *KRE2* belongs to mannosyltransferase gene family and shows α-1,2-mannosyltransferase activity. Like other five members of this family, it consists of a type II membrane proteins which have short cytoplasmic *N*-terminus, a membrane-spanning region and a highly conserved catalytic luminal domain (Lussier et al., 1997). It promotes chain elongation of *N*-linked and *O*-linked mannose residues. Yeast kre2 mutants show reduced *N*-

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linked protein glycosylation and have same amount of β-1-6 glucan like normal cells (Hill et al., 1992).

5.3 ECM33: Ecm33 is a glycosyl phosphatidyl Inositol (GPI) plasma membrane protein (PMP). Ecm33 remain localized to cell membrane and have characteristic receptor L-domain. Yeast Ecm33 mutated strains showed increased sensitivity to calcofluor white and weakened cell wall (Lussier et al., 1997; Pardo et al., 2004). Growth of Ecm33 deletion strain showed temperature sensitivity and was hyper-sensitive to oxidative damage (Terashima et al., 2003). In *C. albicans*, Ecm33p has been reported to be present in abundance and have important role in cell wall integrity and virulence (Gil-Bona et al., 2016).

6. Targets from Sterol Biosynthesis

Sterol is an important component of eukaryotic plasma membrane required for structure maintenance and functioning of cell. The initial steps involved in synthesis of sterol is conserved till synthesis of squalene epoxide from acetyl-CoA, in all major eukaryotes which belongs to three kingdoms namely animals, plants and fungi. Ergosterol is a type of sterol found in fungi (Dupont et al., 2012). The process of ergosterol synthesis is a complex and multi-step process which starts from acetyl-CoA to squalene and then to ergosterol. Ergosterol synthesis is oxygen requiring process where molecular oxygen is required at multiple steps particularly Erg1, Erg3, Erg5, Erg11, Erg25 (Synnott et al., 2010; Gleason et al., 2011). More energy is consumed during ergosterol synthesis as compared to cholesterol (Parks and Casey, 1995).

Ergosterol is a known and well established target for antifungals as three main classes of antifungals namely azoles, polyenes and allylamines, directly or indirectly affect ergosterol (Stylianou et al., 2014; Anand et al., 2015). The advantage of targeting ergosterol and its biosynthesis components for antifungal development is its distinct structure from mammalian cholesterol. Also, their synthesis mechanism is well understood and easy to target (Sangamwar et al., 2008).

7. Few Members of Ergosterol Pathway Which Could Serve as Drug Target

7.1 ERG1: Erg1 is squalene epoxidase enzyme and catalyses conversion of squalene to 2,3-oxidosqualene during ergosterol biosynthesis (Tsai et al., 2004). In *S. cerevisae*, null mutant of Erg1 is viable only under anaerobic condition with exogenous supply of ergosterol (Landl et al., 1996). ScErg1p present inside endoplasmic reticulum only, have enzymatic activity while other Erg1p present inside cell have no activity (Leber et al., 1998). CgErg1p mutant, formed by transposon mutagenesis, has shown increased susceptibility to fluconazole, itraconazole and terbinafine while decreased susceptibility to amphotericin B (Tsai et al., 2004).

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7.2 ERG3: CgErg3p is a $\Delta^{5,6}$ sterol desaturase enzyme which forms double bond between C-5 and C-6 in episterol, during ergosterol pathway (Geber et al., 1995). Deletion of *ERG3* resulted in accumulation of ergosta-7, 22-dien-3β-ol in mutants. *C. glabrata* erg3 deletion mutant has shown increased susceptibility for antifungal. *ERG3* deletion mutant in *S. cerevisiae* has resulted in accumulation of ergosta-7, 22-dien-3β-ol which is responsible for antifungal resistance (Arthington et al., 1991; Watson et al., 1998). In *S. cerevisiae*, erg3 deletion supresses the erg11 null phenotype and results in resistance to azoles with increased viability in aerobic conditions (Taylor et al., 1983; Kenna et al., 1989; Bard et al., 1993).

7.3 ERG5: It is a C-22 sterol desaturase enzyme. It requires molecular oxygen for its activity. It catalyses the double bond formation reaction at C-22 in sterol side chain during conversion of 5,7,24,(28)-Ergosta-trienol to 5,7,22,24,(28)-Ergosta-tetraenol (Figure 1). Since it requires oxygen for its activity, hence hypoxia would affect its working. Also, Erg5p level decreases under hypoxia because Erg5p requires heme and since heme synthesis is an oxygen dependent process (Gleason et al., 2011). It has been reported that *C. albicans ERG5* have no homolog in humans. Thus, it could be a good target for antifungal drug development (Akins, 2005).

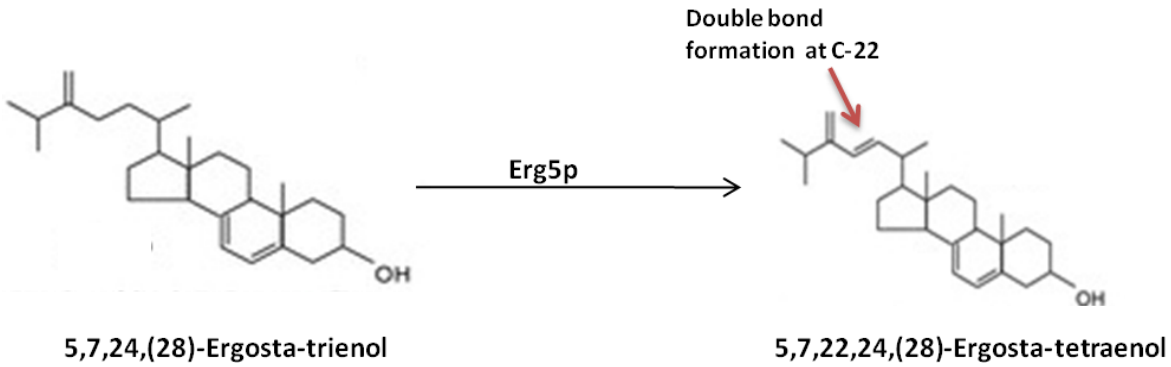

Figure 1. Step of ergosterol biosynthesis pathway catalyzed by Erg5p

7.4 ERG11: *ERG11* encodes for 14α-methyl sterol demethylase which depends on P450. The erg11deletion mutant in *S. cerevisiae* survive under anaerobic conditions in growth media supplemented with ergosterol, ergosta-5, 7, 22-trien-3β-ol (Bossche, 1985; Bossche et al., 1987; Kalb et al., 1987). The enzyme encoded by *ERG11* is the target of azole drugs and point mutation in *ERG1* or over-expression of *ERG11* leads to azole resistance (Nakayama et al., 2001).

7.5 ERG25: It encodes for a C-4 methyl sterol oxidase and removes methyl group from intermediates in first three steps during ergosterol synthesis pathway (Bard et al., 1996). ERG25 deletion mutants in yeast are auxotropic to ergosterol and have accumulated methylated sterol intermediate (Bard et al., 1996). The sterol auxotrophy in erg25 null

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mutants has been reported to get suppressed in erg11 and reduce cellular heme levels (Gachotte et al., 1997).

8. Targets from Membrane Transporters

8.1 CDR1: Two major classes of multidrug transporters or efflux proteins are ATP-Binding Cassette (ABC) and Major Facilitator Superfamily (MFS). ABC transporter is an important class of multi-drug transporter. These efflux pumps play major role in drug resistance particularly resistance to azole, by over-expression of themselves. These transporters do not allow accumulation of drug molecule in pathogen and contribute towards drug resistance.

C. glabrata CDR1 (*Candida* drug resistance) is member of ABC transporter and its increased expression is responsible for *C. glabrata* resistance to azoles (Sanglard et al., 1999). Cdr1 and Cdr2 are two transporters in *Candida*, where Cdr1 is of utmost importance in drug resistance (Prasad et al., 2015). Cdr1p have multiple drug binding sites. The expression of *CDR1* is regulated by Pdr1. *C. glabrata* Cdr1 is closely related to *S. cerevisiae* Pdr5 (Paul and Moye-Rowley, 2014). Cdr1 have a Nucleotide Binding Domain (NBD) followed by transmembrane domain which consist of 6 putative α-helics, at N-terminus and C-terminus (Golin et al., 2007; Prasad et al., 2015). Out of 19 members of ABC superfamily, Cdr1 and Cdr2 have well reported role in drug resistance in *Candida* (Prasad and Goffeau, 2012).

8.2 ATM1: *ATM1* is uncharacterized in *C. glabrata* and is orthologous to *ATM1* of *S. cerevisiae*. In *S. cerevisiae*, Atm1 is a mitochondrial inner membrane protein and belongs to ABC transporter family. Its primary function is to export the mitochondrially synthesized precursor of iron/sulfur (Fe/S) cluster precursor (Kispal et al., 1999). Fe/S cluster containing proteins have role in electron transfer reactions and in many metabolic reactions (Johnson, 1998; Kispal et al., 1999).

In yeast, atm1 deleted cells are sensitive to oxidative stress and have accumulated iron in their mitochondria (Kispal et al., 1997). Null mutants of atm1 are inviable in aerobic condition while slow growing in anaerobic condition (Leighton and Schatz, 1995).

9. Targets from Transcription Factors

Following are few important transcription factors which could be targeted for antifungal drug development.

9.1 UPC2B: *UPC2B* belongs to a very important class of Zn-Cys binuclear cluster protein and exclusively reported in fungi (MacPherson et al., 2006; Clarke et al., 2013). The role of Sterol Regulatory Element Binding Protein (SREBP) has been replaced by Upc2 in members of Saccharomycotina. Upc2 is a transcriptional regulator of ergosterol biosynthesis pathway. Upc2 is a paralog of Ecm22 in *S. cerevisiae*. Both of them remain localized to ER membrane with their transmembrane domain and when sterol level drops, DNA binding domain of Upc2 and Ecm22 relocalizes to nucleus for target gene activation (Marie et al., 2008). Upc2 of *S.*

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cerevisiae have two functional homologs in *C. glabrata*, Upc2A and Upc2B. CgUpc2A is an activator of ergosterol biosynthetic genes and its deletion result in azole sensitivity (Nagi et al., 2011). CgUpc2B is orthologous to Ecm22 and respond only to reduce sterol level while CgUpc2A respond to both reduced level of sterol and heme (Grahl and Cramer Jr, 2010).

9.2 *PDR1:* Pdr1 (Pleiotropic Drug Resistance) is the first transcription factor reported in *S. cerevisiae* responsible for regulating multidrug resistance (Saunders and Rank, 1982). *C. glabrata* Pdr1 regulates transcription of Cdr1, an ABC transporter (Paul et al., 2011). Pdr1 encodes a Zn_2Cys_6 cluster-containing transcription factor (Johnston, 1987). Pdr1 N-terminus contains a cysteine rich Zn-finger required for DNA binding and also have nuclear localization signal (Schjerling and Holmberg, 1996; Delahodde et al., 2001). It activates multidrug resistance genes through Pleiotropic Drug Response Element (PDRE) with a consensus sequence 5'-TCCGCGGA-3' (Mamnun et al., 2002). Pdr1 along with Pdr3 regulate non-ABC transporter along with ABC transporter (DeRisi et al., 2000). Under hypoxic conditions, Pdr1 relocalizes from nuclear membrane to cytosol in very short time (Dastidar et al., 2012).

9.3 ROX1: *ROX1* is transcriptional repressor of hypoxic gene in *S. cerevisiae* and its closest homolog in *C. albicans* is Rfg1, which represses filamentous growth in it (Kadosh and Johnson, 2001). Rox1 inhibit hypoxic gene expression by binding to their promoter along with co-repressor complex, Ssn6-Tup1 (Lowry and Zitomer, 1984; Tzamarias and Struhl, 1995). During hypoxia, heme level drops which inhibit transcription of ROX1 and thus hypoxic genes derepressed (Khalaf and Zitomer, 2001). Rox1 have a 368 amino acid long protein where first $1/4^{th}$ of protein constitute HMG-domain (High Mobility Group) and rest $2/3^{rd}$ constitute repression domain which form repressor complex by binding with Ssn6-Tup1(Balasubramanian et al., 1993; Deckert et al., 1995).

10. Targets from DNA Checkpoint Pathway

DNA checkpoint pathways ensure correct cell cycle progression along with making co-ordination with other pathways like activation of DNA repair, telomere length maintenance, etc. (Zhou and Elledge, 2000; Chiu et al., 2012). The acute DNA damage sensitivity of checkpoint defective cells and the increased cancer susceptibility of organism with checkpoint defects, emphasizes the importance of these surveillance mechanism (Kolodner et al., 2002). DNA checkpoint pathway works by delaying G_1/S transition and blocking G_2/M transition of cell cycle (Putnam et al., 2010). In S phase, when DNA replication encounters a problem like strand break, the ssDNA starts accumulating and it activates two check point pathway one is DNA replication check point which inhibit firing of late replication origin in response to replication stressor and second is intra-S-checkpoint which in response to DNA damage, slows replication and cell cycle progression (Zou and Elledge, 2003; Paulsen and Cimprich, 2007; Putnam et al., 2009). During DNA damage, mitosis is blocked by preventing activation of mitotic Cdk-cyclin (Raleigh and O'Connell, 2000).

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Mec1 and Tel1 are member of Phospho Inositide-3-Kinase like Kinases (PIKK) family and are genetically partially redundant (Sanchez et al., 1999; Szyjka et al., 2008; Putnam et al., 2009). Mec1 and Tel1 are homolog of human ATR and ATM, respectively (Putnam et al., 2010). Mec1 and Tel1 activates downstream target by phosphorylating the serine and threonine residue preceding glutamine in response to DNA damage (Sanchez et al., 1999). Mec1 and Tel1 not only activate check point mediated cell cycle arrest but also help in resuming cell cycle when damage has been cleared (Clerici et al., 2001).

Tel1 and Mec1 have important role in telomere length maintenance by telomerase (Lustig and Petes, 1986; Ritchie et al., 1999). Mrc1 is a component of replication fork and has no identifiable domain or motif, seems to be specific for signaling replication stress (Alcasabas et al., 2001; Osborn and Elledge, 2003). Rad9 activates Chk1 and Rad53 upon DNA damage in Mec1 and Tel1 dependent manner while Mrc1 upon replicative stress (Lee et al., 2003). Pph3 and Psy3 mediate dephosphorylation of Rad53 which is required for restart of replication after DNA damage gets repaired (Keogh et al., 2006; O'Neil et al., 2007).

Dun1 is downstream to Rad53 and gets phosphorylated in response to activation of Rad53. Dun1 upon activation through multiple pathways increases dNTP synthesis, firstly, phosphorylates Sml1 and promotes its degradation, this makes Rnr1 free; secondly, it phosphorylates Dif1 and promotes relocalisation of Rnr2 and finally, it phosphorylates Crt1 leading de-repression of *RNR*2/3/4 transcription (Sanvisens et al., 2013). Finally, a Rnr complex form and starts dNTP formation. RNR are oxygen dependent enzyme containing large R1 and small R2, subunit. R1 is homodimer of Rnr1 while R2 composed of Rnr2 and Rnr4.

11. Conclusion

Several pathways are available in *Candida glabrata*, which may be targeted for antifungal drug developments. Some of the important pathways and specific targets, on the basis of their requirement for viability and virulence of *C. glabrata*, are cell wall biosynthesis (*ecm33*Δ, *kre1*Δ, *kre2*Δ), DNA checkpoint pathway (*dun1*Δ), ergosterol pathway (*erg5*Δ), calcium-calcineurin pathway (*cch1*Δ, *mid1*Δ, *ecm7*Δ), drug transporters (*atm1*Δ, *cdr1*Δ) and transcription factors (*pdr1*Δ, *rox1*Δ, *upc2B*Δ). These targets need further characterization and validation for the development of novel drugs.

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