Am J Transl Res 2020;12(11):6988-7001
www.ajtr.org /ISSN:1943-8141/AJTR0110003

Review Article

Inhibiting roles of farnesol and HOG in morphological switching of *Candida albicans*

Xueting Wang[1*], Hong He[1,2], Jiamei Liu[3*], Shangfeng Xie[1], Jianxin Han[4]

[1]The Affiliated Hospital of Stomatology, School of Stomatology, Zhejiang University, School of Medicine, 395 Yan'an Road, Hangzhou 310006, Zhejiang, China; [2]Key Laboratory of Oral Biomedical Research of Zhejiang Province, Hangzhou 310020, Zhejiang, China; [3]Zhejiang Hospital, Hangzhou 310013, Zhejiang, China; [4]Department of Food Science and Nutrition, School of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310012, Zhejiang, China. [*]Co-first authors.

Received February 28, 2020; Accepted July 18, 2020; Epub November 15, 2020; Published November 30, 2020

Abstract: *Candida albicans* is a major opportunistic fungal pathogen of humans, especially in the oral cavity it involves in precancerous lesions. Numerous transcriptional regulators and hypha-specific genes involved in the morphogenesis mechanisms have been identified. Its virulence is predominantly attributed to the potentiality of morphological switching from yeast and pseudohyphae to hyphal growth. Giving attention in farnesol for prevention or intervention of its virulence sense and possible etiologic role in some uncovered premalignant diseases, in addition, to be a quorum-sensing signal molecule and relationship with HOG pathway, although its morphological switching inhibiting function has attracted high attention and got great progress in being elucidated, their exact mode of action is not completely understood. This report provides a review of characteristic aspects of farnesol signaling and HOG pathway during hyphal development. It also includes other associated pathways, molecules, and novel drug development based on the latest researches over the last decade. Furthermore, farnesol as immunomodulatory to host is an important inferring.

Keywords: Morphological switching, inhibitor, farnesol, HOG pathway, *Candida albicans*, dimorphic switch, immune modulation

Introduction

Human's opportunistic pathogen *Candida albicans* (*C. albicans*) is involved in superficial and systemic diseases such as mucosal premalignant disorders in some population and life-threatening disseminated invasive infections in susceptible individuals [1]. And its yeast-to-hyphal transition and biofilm formation are the predominant virulence-associated traits. Now due to the contribution of some extensive investigations and detailed demonstrations [2-5] about environmental sensing regulation of morphological switching in *C. albicans*, as well as the development of molecular genetic technologies and the first genome declaration in the world, people have come to further recognize its special dimorphism virulence, which is proposed as an etiologic and therapeutic target [6]. Also, numerous key transcriptional regulators and hypha-specific genes (HSGs) involved in the morphogenesis mechanisms have been identified. The morphological switching of *C. albicans* includes hyphal initiation, hyphal maintenance, and hypha-to-yeast transition, and its regulatory mechanisms remain in focus [7]. As the drug resistance of fungal or bacteria increases gradually, the research on these mechanisms is conducive to the development of novel antibacterial agents or antibacterial adjuvants.

The first identified quorum sensing molecule (QSM) farnesol [8] and HOG (high osmolarity and glycerol) pathway [9], as the morphological switching inhibitors, have attracted high attention and achieved great progress [10, 11], their exact mode of action is not completely understood. Here we review the characteristical aspects of farnesol signaling and HOG pathway during hyphal development, as well as the recently acknowledged other associated path-

ways and molecules. Besides, we also reviewed their related applications and the research progresses in antifungal therapy.

Farnesol, a complex role in quorum sensing and switching inhibitor

Quorum sensing molecules allow micro-organisms to monitor their growth and control cell density-dependent phenomena. Studies on the roles of farnesol in *C. albicans* have made progress. Farnesol is secreted by *C. albicans* white cells only whereas opaque and anaerobic cells turn off farnesol synthesis [4], as a side product of the ergosterol biosynthesis pathway by dephosphorylation of farnesol pyrophosphate, primarily mediated by the pyrophosphatase Dpp3 and *C. albicans* produces high amounts of farnesol [10, 12]. Farnesol inhibits filamentation [12] including and hyphal initiation [5] and filamentous-growth [3, 4] without disrupting growth rate. Farnesol blocks the yeast-to-hypha transition at least for a period of 6-10 h after germ tube formation but does not block preexisting hyphal elongation [13]. Farnesol plays an additional role in protecting fungus against oxidative stress [14] and immune modulation [12]. Furthermore, several signaling pathways and molecules are involved in farnesol-mediated mechanisms (**Figure 1**).

Inhibiting the Ras1-Cyr1/cAMP-PKA signal pathway

The inhibitory function of farnesol on the Ras1-Cyr1/cAMP-PKA cascade [3, 12] is well investigated. Ras1p has an advantage of localizing to the membrane and interact with farnesol [15]. The catalytic domain (leucine-rich repeat, LRR) of adenylate cyclase (Cyr1), which once bounds to peptidoglycans will result in filamentation, makes Cyr1p as the central molecular sensor [16]. Therefore, both Ras1p and Cyr1p can be a sensor of farnesol. Farnesol promotes the cleavage of Ras1, resulting in a soluble Ras1 form with a reduced ability to activate Cyr1 [17], and interacts with Ras1 to form a farnesylated Ras1 protein [18]. Furthermore, farnesol directly inhibits Cyr1 activity by binding to the cyclase domain of Cyr1, disturbing cAMP signaling, thus farnesol represses filamentation and supports the hypha-to-yeast transition [17]. The supplementation of exogenous cAMP into media containing farnesol completely restored filamentation [3, 19]. Cyr1 can integrate a diverse range of external signals [20], generating a pulse of cAMP essential for hyphal initiation. Studies by Lu et al. [21] indicated the activation of the cAMP-dependent protein kinase A (PKA) pathway was required to down-regulate the transcription of *NRG1* during hyphal growth as Nrg1 protein level did not show an obvious reduction in *cyr1* and *tpk2* mutants. Tpk1 and Tpk2 are PKA catalytic subunits, and deletion mutants of *TPK2* block hyphal formation [22, 23]. The mutants lacking *pde2* showed increased cAMP signaling and more resistant to farnesol-mediated induction of hypha-to-yeast transitions [24]. *CYR1* and *PDE2* regulate a pair of enzymes associated with cAMP synthesis and degradation, and *PDE2* regulation by farnesol was subordinate to *CYR1* regulation [25]. In addition, farnesol can induce hydrogen peroxide resistance. Strains lacking either Ras1 or Cyr1 would no longer exhibit increased protection against hydrogen peroxide upon preincubation with farnesol [14], demonstrating that farnesol induces oxidative-stress resistance *via* Ras1-Cyr1 signaling pathway.

Efg1, a key transcriptional activator of HSGs, is activated through the Ras-cAMP signal transduction pathway to induce hyphal growth [26]. Thus, farnesol blocks activation of the HSGs regulated by Efg1 by inhibiting the Ras-cAMP pathway. However, farnesol does not affect the *EFG1* mRNA level. Langford et al. [27] identified that Czf1 played an essential role as downstream of Efg1 in the response to farnesol since the mutation in *CZF1* was resistant to farnesol, Efg1 was required for filamentation in aerobic condition while Czf1 was required in hypoxia. *CZF1* expression is regulated by Efg1 and negatively regulated by Czf1 [28].

Blocking Cup9 degradation to inhibit the Sok1-mediated degradation of Nrg1

Nrg1 is a transcriptional regulator and the major repressor of hyphal development by binding to the DNA with the Tup1 complexes [4]. By chromatin immunoprecipitation of C-terminal Myc-tagged Nrg1, Lu et al. [21] found that Nrg1 was binding to the promoters of HSGs including *HWP1*, *ALS3*, and *ECE1* during yeast growth. As the level of Nrg1 repressor decreases, less Nrg1 occupancy at hypha-specific gene promoters [20]. Farnesol inhibits hyphal initiation

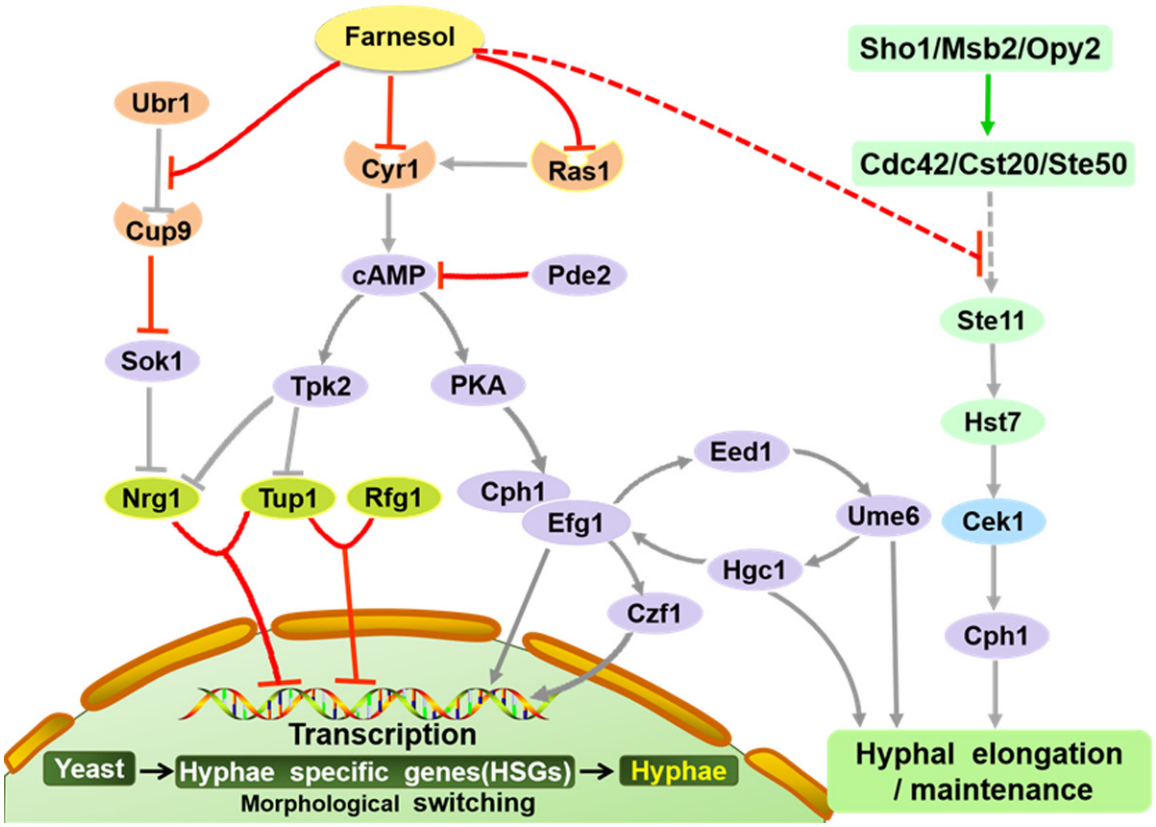

Figure 1. Morphological switching inhibitor, farnesol signaling in *Candida albicans*. Arrows indicate stimulation, T-shaped lines indicate inhibition, and gray lines represent relationships that are inactive.

mainly through blocking the protein degradation of Nrg1 [5]. Lu et al. identified Sok1 as a kinase required for Nrg1 degradation during inoculation, and overexpression of *SOK1* overcame the farnesol-mediated inhibition of hyphal initiation. They found Cup9 was responsible for the repression of *SOK1* expression and farnesol inhibited Cup9 degradation mediated by Ubr1 [5]. By the way, hyphal initiation requires both the cAMP-PKA pathway-dependent transcriptional down-regulation of *NRG1* and Sok1-mediated degradation of Nrg1 protein, neither pathway alone is enough for hyphal initiation [5].

Increasing Tup1 levels

Tup1, a transcriptional repressor, functions with the DNA binding proteins Nrg1 and Rfg1 to inhibit the HSGs expression, negatively regulating the yeasts to hyphae transition [4]. Tup1 is a crucial component in response to farnesol in *C. albicans*, as the *tup1/tup1* and nrg1/nrg1 mutants, not the *rfg1/rfg1* mutant, fails to respond to farnesol [4]. Interestingly, they [4] also observed that *tup1* and *nrg1* mutants elevated farnesol synthesis 17~19 fold. In addition, farnesol causes a small but consistent increase of *TUP1* mRNA, a ~2.5-fold increase in Tup1 protein level [18], and even the expression of two Tup1-regulated HSGs, *HWP1* and *RBT1*.

Regulating MAP kinase pathway

Farnesol was considered to function through the Cek1-MAPK pathway to inhibit morphological switching, since the addition of farnesol represses the expression of *CPH1*, *HST7* and *GAP1* [2] but *CST20* was not affected [29]. Some researchers found *C. albicans* mutants lacking the histidine kinase Chk1 were refractory to the inhibitory effect of farnesol [30], Farnesol acts on two-component signaling *via* Chk1.

Correlation between farnesol and EED1

EED1 (Epithelial Escape and Dissemination 1), a unique gene of *C. albicans*, plays a crucial

role in the expression of the hyphy-specific genes, the extension of germ tubes into elongated hyphae and hyphal maintenance [31]. Expression of *EED1* is regulated by the transcription factor Efg1 and is negatively regulated by Nrg1 and Tup1 as well [26]. Farnesol might reduce *EED1* expression mediated by Nrg1, promoting lateral yeast formation and the hypha-to-yeast transition [12]. Ume6 is a transcription factor that controls the expression of a wide range of hyphal-associated genes (HAGs), affects biofilm formation, and promotes hyphal development [32]. The expression of *UME6* is affected by *EED1* [26] and supports the maintenance of *HGC1* expression, which is involved in hyphal maintenance. Polke et al. [12] have identified the first mutant (*EED1*Δ/Δ) with hypersecretion of farnesol and hypersensitivity to farnesol, which contributes to the reverse morphogenesis and is not affected by hyperactivation of the RAS1-cAMP-pathway, or deletion of Czf1 and Nrg1, thus farnesol might acts on *EED1 via* a novel mechanism associated with a regulated farnesol transporter such as Ste3p, Ste6p or another unidentified yet, which still need further researches [13]. The mutants (*EED1*Δ/Δ) still can form germ tubes to invade epithelium but fail to elongate filaments, and the lateral yeast formation from preexisting hyphae is observed [10, 26]. The *C. albicans* mutant (*EED1*Δ/Δ) is a good study model for the investigation on fungal dimorphism as well as the characteristics of farnesol in *C. albicans*.

Farnesol as an immunomodulatory molecule

Abe et al. indicated farnesol decreased murine macrophages phagocytic and anti-Candida activity *in vitro* with nuclei morphological change and DNA fragmentation, perhaps *via* induction of ROS [33]. Hargarten et al. [34] discovered that *C. albicans* white cells secrete farnesol as a potent chemoattractive stimulator of macrophages, resulting in an 8.5-fold increase in macrophage migration *in vitro* and a 3-fold increase in the peritoneal infiltration of macrophages *in vivo*. Farnesol induces the activation of neutrophils and monocytes and promotes oxidative burst and the release of proinflammatory cytokines TNF-α and MIP-1α, without enhanced fungal phagocytosis or killing [35]. The study by Zawrotniak et al. showed that farnesol activated the neutrophil extracellular traps (NETs) production, and the Mac-1 and TLR2 receptors were responsible for farnesol recognizing, promoting neutrophils to release ROS quickly after activation by farnesol [36]. In addition, farnesol significantly affects the differentiation of monocytes into immature dendritic cells (iDC) and the ability of iDC to induce proper T cell responses, thereby mitigating the Th1 response [35].

Although farnesol plays a protective role in superficial *C. albicans* infections such as oral candidiasis [37] with decreasing filamentation rates, less tissue damage and a protective epithelial response, farnesol acts as a virulence factor in systemic infection possibly by modulating immune surveillance towards a non-protective Th2 response, promoting tissue damage and the dispersion of yeast cell [10]. Farnesol modulates human dendritic cells (DC) function *via* multiple signaling pathways during DC maturation to overcome DC-mediated immunity surveillance. Farnesol increases the expression of the Ag-presenting glycoprotein CD1d by activating nuclear receptors PPARγ and RARα, as well as p38 MAPK. Farnesol reduces IL-12 secretion while enhances the release of IL-10 *via* MAPK and NF-κB signaling pathways, impairing their ability to activate invariant NKT, Th1, and $FOXP3^+$ regulatory T cells [38]. Exposing *C. albicans* to acidic environments results in the exposure of cell wall pathogen-associated molecular patterns (PAMPs, including β-glucan and chitin) and increases the detection of the yeast by macrophages. Then farnesol regulates the remasking of β-glucan, while chitin remasking is regulated by Efg1 through regulation of CHT2, influencing the host's innate immune responses [39]. Disrupting the masking of surface markers so that they can be recognized by the innate immunity of the host can be one of the follow-up research directions. Farnesol promotes intestinal epithelial barrier function by activating JAK/STAT3 signaling pathway in differentiated Caco-2 cells and inducing the Zonula Occludens-1 Protein (ZO-1) expression [40]. The immunomodulatory mechanisms still need more further studies.

Farnesol-mediated apoptosis

The influence of farnesol on *C. albicans* yeast cells is concentration-dependent, while higher

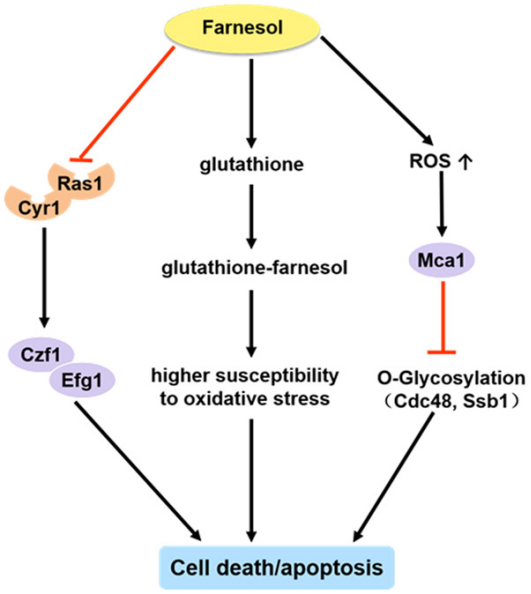

Figure 2. Farnesol-mediated cell death. Red T-shaped lines indicate inhibition.

farnesol level (more than 200 μM) is stressful for yeasts, lower level (about 40 μM) protects them from stress [41, 42]. It has been already proved that farnesol promotes apoptosis (**Figure 2**).

Langford et al. discovered Efg1 and Czf1 coordinate the farnesol-mediated cell death in *C. albicans* synergistically [27]. Farnesol might trigger apoptosis *via* accumulation of reactive oxygen species (ROS) which activates intracellular caspases and damages essential cellular compartments but the mechanisms are still unclear [10, 41]. Zhu et al. [43] demonstrated farnesol conjugated with intracellular glutathione combined with Cdr1p-mediated extrusion of glutathione-farnesol complexes, resulting in glutathione depletion, higher susceptibility of the cell to oxidative stress and ultimately cell apoptosis. The only metacaspase Mca1 of *C. albicans* is required and activated during farnesol-induced cell apoptosis by affecting the glycosylation of several critical proteins such as Cdc48 and Ssb1 [44]. Edc3 is a key regulator of CaMca1 expression [45]. It was observed that the expression of the apoptosis genes (*CARD-9* and *NOXa*) increased and the expression of the anti-apoptosis genes (Bcl-2) decreased in the farnesol-treated Galleria mellonella [46]. Farnesol also has anti-inflammatory and anticancer properties by modulating Ras protein and NF-κB activation to downregulate the expression of COX-2, TNF-α, IL-6, and modulating various tumorigenic proteins and signal transduction cascades [47-49]. Farnesol inhibits tumor growth in multiple myeloma by modulating the signal transducer transcriptional activator 3 (STAT3) pathway [48].

The effects of farnesol on polymicrobial biofilms and other species

C. albicans can establish and persist complex polymicrobial biofilms with other different human microbial pathogens, including *Staphylococcus aureus* (*S. aureus*), *Streptococcus mutans* (*S. mutans*) *etc.* presenting enhanced pathogenicity and drug resistance by impeding drug penetration and immune cell access. Farnesol activates drug efflux pumps in *S. aureus*, enhancing tolerance to antibacterials [50, 51]. Farnesol might inhibit staphyloxanthin biosynthesis by binding to the CrtM enzyme competitively and enhance tolerance to oxidative stressors by activating the thiol-based oxidative-stress response [52]. However, the effect of farnesol is a dose-dependent manner. Farnesol at high concentrations inhibits the growth of *S. aureus* and induce the leakage of potassium ions, causing the cytoplasmic membrane of *S. aureus* to be disordered and eventual decomposed [53]. A 30 mM concentration of farnesol appears to reduce the rate of colonization and inhibit biofilm formation of *S. aureus in vitro* and *in vivo* [54].

A study [42] found that 25-50 μM farnesol enhanced S. mutans Gtf activity, promoting microcolony development and biofilm formation. Whereas Cao [55] reported 500-1000 μM farnesol inhibited biofilm formation by 26.4% to 37.1% and downregulated the expression of virulence-associated genes including luxS, brpA, ffh, recA, nth, and smx. And when the concentrations above 12.5 mM, farnesol behaves similarly to chlorhexidine gluconate [56]. Farnesol at 0.78 mM and 1.56 mM significantly reduced the acid production of S. mutans biofilm [57]. Rocha [58] reported that the water-soluble exopolysaccharide in the extracellular matrix (ECM) was significantly reduced in the combination therapy of myricetin, farnesol, and fluoride. Cernakova [59] determined 200 μM farnesol was the minimum inhibitory concentration that inhibited the growth of *C. albicans*

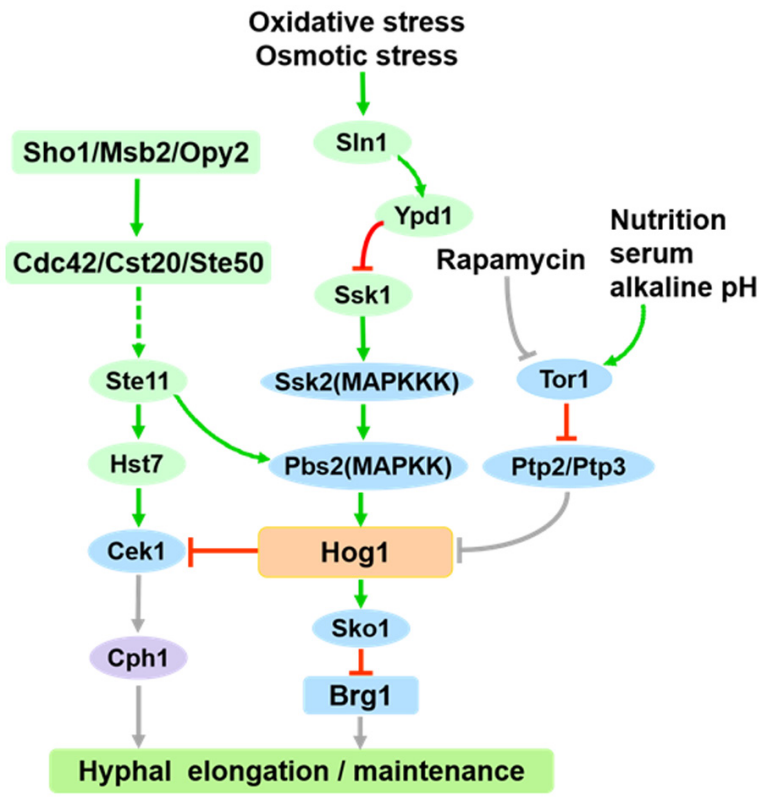

Figure 3. Morphological switching Inhibitor, HOG pathways in *Candida albicans*. Arrows indicate stimulation, T-shaped lines indicate inhibition, and gray lines represent relationships that are inactive.

SC5314 biofilm by 50%, and observed *C. albicans* mainly existed as blastospores but the growth of S. mutans was not influenced in a dual biofilm with the addition of 200 μM farnesol.

Egbe et al. [60] found that farnesol reduced the 48S preinitiation ribosomal complex levels in *Saccharomyces cerevisiae* by affecting the interaction of the mRNA with the small ribosomal subunit, inhibiting the initiation step of translation and ultimately the filamentous growth. Farnesol inhibits *Fusarium keratoplasticum* biofilm formation by destructing hyphae and the extracellular matrix in preformed biofilm and preventing conidia adhesion and filamentation [61].

HOG pathway responsible for repression of the yeast-to-hypha transition

The Hog pathway in *C. albicans*, mediated by the MAPK Hog1, plays a central role in the response and adaptation to osmotic and oxidative stress and is also involved in morphogenesis, cell wall biogenesis, and virulence. And Hog1 plays a repressive role in hyphal development during hyphal elongation [11] (**Figure 3**).

The HOG MAPK pathway

Hog1 participates in two distinct morphogenetic processes, as a repressor (yeast-to-hypha transition) and as an inducer (chlamydospore formation) [62]. In contrast to the Hog1 pathway in *Saccharomyces cerevisiae*, Cheetham et al. [63] demonstrated that Hog1 in *C. albicans* was regulated by a single Ssk2. And Ssk2 regulates Hog1 *via* activation of Pbs2. Annotation of the *C. albicans* genome sequence [63] revealed four putative osmosensor proteins: Chk1, Nik1 (Cos1), Sln1, and Ssu81 (Sho1). In *C. albicans*, the *SLN1* branch is responsible for the activation of Hog1 upon oxidative stress. Sho1p activates Pbs2p *via* Ste11p [64]. A stimulus is perceived by the Sln1 sensor at the plasma membrane, the signal is transmitted to the MAPK central core *via* the Sln1-Ypd1-Ssk1 signaling branch, triggering the MAPKKK Ssk2 activation, which sequentially phosphorylates the MAPKK Pbs2 and, consequently, the MAPK Hog1 [11]. Phosphorylated Hog1 is repressive to hyphal elongation. Sln1 and Nik1 are thought as upstream of Chk1 [65]. The severely reduced hyphal formation was observed in ssk1 mutants even on serum agar [66]. After phosphorylated transiently upon osmotic stress, Hog1 translocates into the nucleus, regulates gene transcription, and mediates intracellular glycerol accumulation [67]. Hog-mediated repression of the yeast-to-hypha switch is independent of the Efg1 and Cph1 transcription factors [2].

The three mutants with the deletion of *HOG1*, *PBS2*, or *SSK2* showed the strongest hyphal elongation under nutrient-rich conditions, which demonstrates the HOG pathway plays a repressive role on hyphal elongation in *C. albi-*

cans. And the mutants expressed the GATA transcription factor Brg1 to induce hyphal elongation [68]. Phosphorylated Hog1 represses the expression of *BRG1* indirectly but *via* the transcriptional repressor Sko1 [9], *C. albicans* with the deletion of *sko1* reverts virulence [69]. Only a basal level of phosphorylated Hog1 is required for the promoter association of Sko1 to repress the hyphal transcriptional program.

Function in the TOR pathway

The target of rapamycin (TOR) protein kinase pathway is the major nutrient-sensing pathway. *C. albicans* has a single TOR kinase, Tor1, which is rapamycin-sensitive and a central regulator of cell growth in response to nitrogen and amino acid availability. Tor1 can be activated in a nutrient-rich medium inducing hyphae cells to convert to yeast cells [70]. Tor1 inhibits cell adhesion by repressing the expression of adhesin genes *ALS1*, *ALS3*, *HWP1*, and *ECE1* [64]. Diminishing Tor1 activity upon rapamycin or in nutrient-limiting media activates the two Hog1 tyrosine phosphatases, Ptp2 and Ptp3, that down-regulate Hog1 basal activity. The inactivation of Hog1 is important for sustained hyphal elongation. Sko1 dissociates from the *BRG1* promoter when the level of phosphorylated Hog1 is lowered (by rapamycin) or abolished (in *HOG1* deletion mutant), and ultimately activate *BRG1* expression, keeping Nrg1 protein off the promoters of HSGs and inducing sustained hyphal elongation [68, 71]. Besides, it seems that Hog1 phosphorylation in response to stresses plays a dominant role in the regulation *via* Tor1 signaling [68].

Brg1 plays an importation role in hyphal elongation and *BRG1* expression requires both the removal of Nrg1 and a sub-growth inhibitory level of rapamycin [71]. The Nrg1 protein, the major transcription inhibitor of hyphal development, is temporarily removed from promoters of HSGs upon activation of the cAMP/PKA pathway [21]. Nrg1 binding sites are in nucleosome-free regions in yeast cells, whereas Brg1 binding sites are occupied by nucleosomes. Nucleosome disassembly during hyphal initiation exposes the binding sites for Nrg1 and Brg1. But Brg1 recruits the histone deacetylase Hda1 to promoters of HSGs, and Hda1 deacetylates a subunit of the NuA4 histone acetyltransferase module, leading to the eviction of the NuA4 acetyltransferase module, nucleosome repositioning and flocclusion of Nrg1 binding sites that prevents Nrg1 from binding to the promoters. However, it is only when Nrg1 is gone that promoter recruitment of Hda1 for hyphal maintenance happens and Brg1 can bind to the promoters [21]. Furthermore, the hypha-specific regulator Ume6 is a key downstream target of Brg1 and it functions after Brg1 as a built-in positive feedback regulator [68].

Mds3, a regulator of pH-dependent morphogenesis, functions in parallel with the classic Rim101 pH-sensing pathway. As Mds3 Delta/Delta cells have an expression profile indicative of a hyperactive TOR pathway, and the transcriptional and morphological defects of the mutant are rescued by rapamycin, Mds3 is believed to be a new element of the TOR pathway that contributes to morphogenesis in *C. albicans* [72]. Sit4 and Mds3 may function similarly in the TOR pathway due to their coimmunoprecipitation.

Function in the Cek1 pathway

In *C. albicans*, there are four MAPKs identified that play important roles in cell physiology, including Mkc1, Cek1, Cek2, and Hog1. The Cek1-mediated pathway was discovered that implicated in mating, invasive hyphal growth, cell wall formation, virulence, biofilm formation, quorum sensing, and so on. It was reported that Cek1 regulates the β-glucan exposure on the cell wall [64]. The *C. albicans SHO1* branch is involved in the activation of Cek1. The Sho1 adaptor protein links oxidative stress to cell wall biogenesis and morphogenesis, and Shol plays a minor role in response to oxidative stress, which mainly occurs through a putative Sln1-Ssk1 branch of the HOG pathway [73]. Msb2, a mucin-like protein, mediates activation of Cek1 mitogen-activated protein kinase and controls the cell wall construction [74]. Cst20, a protein kinase of the PAK family, belongs to the Cst20-Hst7-Cek1-Cph1 MAPK pathway [75]. The transmembrane proteins Sho1, Msb2, and Opy2 sense external signals [11] and may form a protein complex that may interact with Cdc42, Cst20 and Ste50, transmitting signals to Cek1-mediated pathway and triggering Cek1 phosphorylation. In Addition, Sho1 and Opy2 may play a major role in the formation of the complex, which may be necessary to recruit all the

elements required to polarization/depolarization of the cytoskeleton and other associated structures, such as the septin ring, under osmotic stress [67]. Hog1 represses the activation of the Cek1 MAPK under basal conditions [76], and hog1, pbs2, or ssk2 mutants display enhanced hyphal formation and hyperphosphorylation of Cek1 MAPK, which demonstrate an intrinsic connection between the HOG and the Cek1-mediated pathways [11].

Other signal components and functions

The endosomal sorting complex required for transport (ESCRT) system is involved in hyphal induction in a neutral-alkaline environment *via* the Rim101 pathway, and ESCRT protein Vps4 is essential for polarity maintenance during hyphal formation and the proper localization of Cdc42 and Cdc3. Deletions of the ESCRT proteins cause delayed germination, a decreased recovery rate of GFP-Ras1, and downregulation of HSGs such as *HGC1* [77]. Mu C et al. [78] identified phosphatidate phosphatase Pah1 as a crucial role in hyphal growth and virulence of *C. albicans*, as the *pah1Δ/Δ* mutants display hyphal defects due to the reduced expression of *UME6*.

The development of novel antimicrobial agents

Farnesol could be used as an adjuvant, potential antimicrobial agent to contribute to the prevention of candidiasis and caries with great therapeutic potential. Farnesol also can be found in essential oils [49]. Chitosan (CS) nanogel could be a useful nanocarrier for the pharmaceutical application of farnesol with little cytotoxicity. And CS nanogel containing farnesol decreased the expression of *HWP1* and *SAP6* genes in *C. albicans* [79]. Biosurfactant AC7, a lipopeptide from *Bacillus subtilis*, coats material surfaces in combination with farnesol, contributing to the inhibition of initial adhesion and biofilm growth of *C. albicans* and the prevention of medical device-associated infection [80]. Farnesol isomers, sources of separation, and preparation methods influence compound pharmacological properties and/or toxicological effects directly, which is one of the limitations for future pharmaceuticals development [49].

Farnesol might alter the drug resistance of the resistant *C. albicans* and other microbial species by inhibiting biofilm formation and regulating drug transportation. Farnesol in combination with fluconazole (FLC) decreases the minimal inhibitory concentrations (MIC) of FLC [81] by reducing the efflux activity of both Cdr1 and Cdr2 pumps, the ATP-binding cassette efflux transporters [82], which is dependent on farnesol concentrations and changes the resistance to azoles in the FLC-resistant *C. albicans*. Interestingly, farnesol induced *CDR1* expression by activating the Zn cluster transcription factors Tac1 and Znc1 [83]. Moreover, it was observed that 200 μmol/L farnesol downregulated the expression of the ergosterol genes *ERG20* and *ERG11* [84]. Farnesol showed synergistic effects with fluconazole or 5-flurocytosine against biofilms, but antagonistic effects with terbinafine and itraconazole [85]. The combination of farnesol and carvacrol displayed synergistic inhibitory effects against amphotericin-B-resistant *C. albicans* in single- and mixed-species biofilms [86].

Monteiro [87] and Sebaa [88] suggested farnesol and tyrosol might as novel adjuvants in oral hygiene contributing to the prevention of dental caries and candidiasis with an antibiofilm effect on S. mutans and Candida species. 200 mM tyrosol can cause cell damage and significantly reduce metabolic activity and biofilms formation [89]. Barot [90] fabricated flowable resin composites incorporated with farnesol loaded halloysite nanotubes (Fa-HNTs) successfully without cytotoxicity on mouse embryonic fibroblast cells, and the composite with 7-13 wt% Fa-HNT showed promising physicochemical properties and significant antimicrobial activity, might beneficial for preventing secondary caries. However, long-term antibacterial properties still need further study. Codelivery of farnesol and ciprofloxacin inhibits biofilms of resistant *Pseudomonas aeruginosa* at significantly lower ciprofloxacin concentration *in vitro* [91].

Kalopanaxsaponin A [92], trans-cinnamaldehyde [93], Eucarobustol E [94], Biatriosporin D [95], Shikonin [96] upregulate the expression of Dpp3 to promote farnesol secretion, but further experiments *in vivo* are required. Zerumbone, a monocyclic sesquiterpene derived from Zingiber zerumbet (L.) Smith, decreases hyphal growth and suppresses biofilm forma-

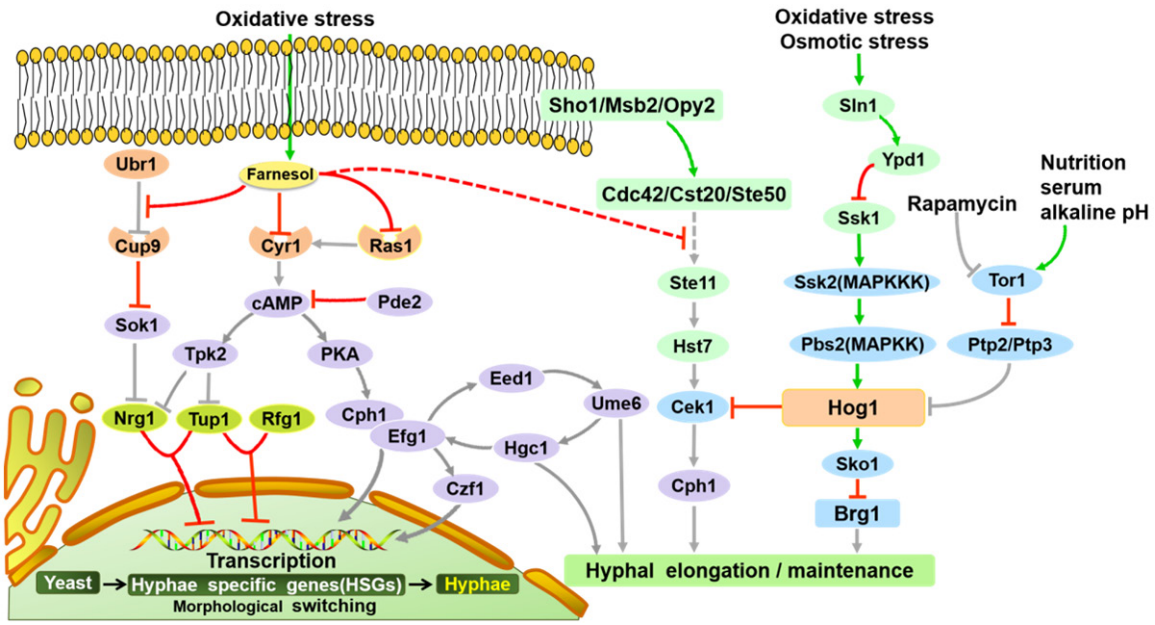

Figure 4. Morphological switching inhibitor farnesol and HOG pathways in *Candida albicans*. Farnesol inhibits hyphal growth by repressing NRG1 and TUP1 transcriptional down-regulation through the Ras1-Cyr1/cAMP-PKA pathway, by blocking the Cup9 degradation to inhibit the Sok1-mediated degradation of Nrg1 and by regulating Cek1-MAPK pathway. The HOG pathway plays a repressive role on filamentation and is also regulated by TOR pathway and inhibits Cek1 function. Arrows indicate stimulation, T-shaped lines indicate inhibition, and gray lines represent relationships that are inactive.

tion by significantly downregulating the expression of biofilm-related genes and HSGs, including *HWP1* and *ALS3* [97]. Moreover, zerumbone remarkably inhibits the dual-species biofilms of *C. albicans* and *S. aureus* [98].

Further explorations on the regulation of farnesol synthesis, potential receptor, and transporters on *C.albicans* are required, beneficial for developing new antifungal drugs that promote more farnesol synthesis, increase the farnesol intercellular concentration and enhance the antifungal effects. The involved molecules above may be potential and effective drug targets of antifungal treatment. The combination of conventional therapeutic agents with new adjuvants such as farnesol might provide effective anti-infective treatment strategies, especially against the multidrug-resistant microorganisms. However, most experiments are only limited to *in vitro*, and further studies *in vivo* and clinical are required to evaluate the efficacy and safety.

Conclusions

Farnesol inhibits *NRG1* and *TUP1* transcriptional down-regulation through the Ras1-Cyr1/cAMP-PKA pathway and the Sok1-mediated degradation of Nrg1 protein to repress hyphal initiation, and inhibits hyphal elongation through Cek1-MAPK pathway. The HOG MAPK pathway in *C. albicans* is not only an osmosensing pathway, but also involved in regulating cell wall biogenesis and morphogenesis, playing a repressive role on filamentation. The activated Hog1 is also regulated by TOR pathway and inhibits Cek1 function (**Figure 4**). Multiple and intricate signal transduction pathways in *C. albicans* interact and coordinate with each other under different conditions, which are relative to its pathogenesis. Although the network has never been annotated so exhaustively, researchers have identified more signal molecules and pathways involved in morphogenesis and get more knowledgeable about the network, extraordinarily how morphologic switching and virulence or difference are regulated in or between vivo and vitro. An increased understanding of the molecular mechanism of morphologic inhibitors benefits research on pathological mechanisms and development of clinical therapy and novel antifungals.

Acknowledgements

This work was supported by the Education Reform Program (yxyb20172030) from the

School of Medicine, Zhejiang University in China, the Nonprofit Specific Fund (2015020-18) from the National Hygiene and Health Commission of China, the Co-construction Program (WKJ-ZJ-1623) from the Provincial Bureau and National Commission of Hygiene & Health in China, and the National key research and development program (2016YFC0902702).

Disclosure of conflict of interest

None.

Abbreviations

C. albicans, Candida albicans; cAMP, cyclic adenosine monophosphate; ChIP, chromatin immunoprecipitation; CPH1, Candida Pseudo Hyphal regulator; CS, Chitosan; Czf1, *C. albicans* zinc finger 1; DC, dendritic cells; ECM, the extracellular matrix; *EED1*, Epithelial Escape and Dissemination 1; ESCRT, the endosomal sorting complex required for transport; Fa-HNTs, farnesol loaded halloysite nanotubes; FLC, fluconazole; HAGs, hyphal-associated genes; HOG, high-osmolarity and glycerol; HSGs, hypha-specific genes; iDC, immature dendritic cells; LRR, leucine-rich repeat; MAPK, mitogen-activated protein kinase; MAPKK, mitogen-activated protein kinase kinase; MAPKKK, mitogen-activated protein kinase kinase kinase; MIC, the minimal inhibitory concentrations; MIP-1α, macrophage inflammatory protein 1 alpha; mRNA, messenger Ribonucleic Acid; NETs, the neutrophil extracellular traps; PAMPs, pathogen-associated molecular patterns; PKA, protein kinase A; QSM, quorum sensing molecule; ROS, reactive oxygen species; STAT3, the signal transducer transcriptional activator 3; TNF-α, tumor necrosis factor alpha; TOR, target of rapamycin; ZO-1, the Zonula Occludens-1.

Address correspondence to: Dr. Hong He, The Affiliated Hospital of Stomatology, School of Stomatology, Zhejiang University, School of Medicine, 395 Yan'an Road, Hangzhou 310006, Zhejiang Province, China. Tel: +86-0571-87217431; Fax: +86-0571-8721-7423; E-mail: honghehh@zju.edu.cn

References

[1] Yapar N. Epidemiology and risk factors for invasive candidiasis. Ther Clin Risk Manag 2014; 10: 95-105.

[2] Biswas S, Van Dijck P and Datta A. Environmental sensing and signal transduction pathways regulating morphopathogenic determinants of Candida albicans. Microbiol Mol Biol Rev 2007; 71: 348-376.

[3] Hall RA, Cottier F and Muhlschlegel FA. Molecular networks in the fungal pathogen Candida albicans. Adv Appl Microbiol 2009; 67: 191-212.

[4] Kebaara BW, Langford ML, Navarathna DH, Dumitru R, Nickerson KW and Atkin AL. Candida albicans Tup1 is involved in farnesol-mediated inhibition of filamentous-growth induction. Eukaryot Cell 2008; 7: 980-987.

[5] Lu Y, Su C, Unoje O and Liu H. Quorum sensing controls hyphal initiation in Candida albicans through Ubr1-mediated protein degradation. Proc Natl Acad Sci U S A 2014; 111: 1975-1980.

[6] Jacobsen ID, Wilson D, Wachtler B, Brunke S, Naglik JR and Hube B. Candida albicans dimorphism as a therapeutic target. Expert Rev Anti Infect Ther 2012; 10: 85-93.

[7] Jacobsen ID and Hube B. Candida albicans morphology: still in focus. Expert Rev Anti Infect Ther 2017; 15: 327-330.

[8] Hornby JM, Jensen EC, Lisec AD, Tasto JJ, Jahnke B, Shoemaker R, Dussault P and Nickerson KW. Quorum sensing in the dimorphic fungus Candida albicans is mediated by farnesol. Appl Environ Microbiol 2001; 67: 2982-2992.

[9] Alonso-Monge R, Roman E, Arana DM, Prieto D, Urrialde V, Nombela C and Pla J. The Sko1 protein represses the yeast-to-hypha transition and regulates the oxidative stress response in Candida albicans. Fungal Genet Biol 2010; 47: 587-601.

[10] Polke M, Leonhardt I, Kurzai O and Jacobsen ID. Farnesol signalling in Candida albicans - more than just communication. Crit Rev Microbiol 2018; 44: 230-243.

[11] Roman E, Correia I, Prieto D, Alonso R and Pla J. The HOG MAPK pathway in Candida albicans: more than an osmosensing pathway. Int Microbiol 2020; 23: 23-29.

[12] Polke M, Sprenger M, Scherlach K, Alban-Proano MC, Martin R, Hertweck C, Hube B and Jacobsen ID. A functional link between hyphal maintenance and quorum sensing in Candida albicans. Mol Microbiol 2017; 103: 595-617.

[13] Nickerson KW and Atkin AL. Deciphering fungal dimorphism: farnesol's unanswered questions. Mol Microbiol 2017; 103: 567-575.

[14] Deveau A, Piispanen AE, Jackson AA and Hogan DA. Farnesol induces hydrogen peroxide resistance in Candida albicans yeast by inhibiting the Ras-cyclic AMP signaling pathway. Eukaryot Cell 2010; 9: 569-577.

[15] Shchepin R, Dumitru R, Nickerson KW, Lund M and Dussault PH. Biologically active fluorescent farnesol analogs. Chem Biol 2005; 12: 639-641.
[16] Xu XL, Lee RT, Fang HM, Wang YM, Li R, Zou H, Zhu Y and Wang Y. Bacterial peptidoglycan triggers Candida albicans hyphal growth by directly activating the adenylyl cyclase Cyr1p. Cell Host Microbe 2008; 4: 28-39.
[17] Piispanen AE, Grahl N, Hollomon JM and Hogan DA. Regulated proteolysis of Candida albicans Ras1 is involved in morphogenesis and quorum sensing regulation. Mol Microbiol 2013; 89: 166-178.
[18] Gupta P, Sharma M, Arora N, Pruthi V and Poluri KM. Chemistry and biology of farnesol and its derivatives: quorum sensing molecules with immense therapeutic potential. Curr Top Med Chem 2018; 18: 1937-1954.
[19] Davis-Hanna A, Piispanen AE, Stateva LI and Hogan DA. Farnesol and dodecanol effects on the Candida albicans Ras1-cAMP signalling pathway and the regulation of morphogenesis. Mol Microbiol 2008; 67: 47-62.
[20] Hogan DA and Muhlschlegel FA. Candida albicans developmental regulation: adenylyl cyclase as a coincidence detector of parallel signals. Curr Opin Microbiol 2011; 14: 682-686.
[21] Lu Y, Su C, Wang A and Liu H. Hyphal development in Candida albicans requires two temporally linked changes in promoter chromatin for initiation and maintenance. PLoS Biol 2011; 9: e1001105.
[22] Bockmuhl DP, Krishnamurthy S, Gerads M, Sonneborn A and Ernst JF. Distinct and redundant roles of the two protein kinase A isoforms Tpk1p and Tpk2p in morphogenesis and growth of Candida albicans. Mol Microbiol 2001; 42: 1243-1257.
[23] Sonneborn A, Bockmuhl DP, Gerads M, Kurpanek K, Sanglard D and Ernst JF. Protein kinase A encoded by TPK2 regulates dimorphism of Candida albicans. Mol Microbiol 2000; 35: 386-396.
[24] Lindsay AK, Deveau A, Piispanen AE and Hogan DA. Farnesol and cyclic AMP signaling effects on the hypha-to-yeast transition in Candida albicans. Eukaryot Cell 2012; 11: 1219-1225.
[25] Chen S, Xia J, Li C, Zuo L and Wei X. The possible molecular mechanisms of farnesol on the antifungal resistance of C. albicans biofilms: the regulation of CYR1 and PDE2. BMC Microbiol 2018; 18: 203.
[26] Martin R, Moran GP, Jacobsen ID, Heyken A, Domey J, Sullivan DJ, Kurzai O and Hube B. The Candida albicans-specific gene EED1 encodes a key regulator of hyphal extension. PLoS One 2011; 6: e18394.
[27] Langford ML, Hargarten JC, Patefield KD, Marta E, Blankenship JR, Fanning S, Nickerson KW and Atkin AL. Candida albicans Czf1 and Efg1 coordinate the response to farnesol during quorum sensing, white-opaque thermal dimorphism, and cell death. Eukaryot Cell 2013; 12: 1281-1292.
[28] Vinces MD, Haas C and Kumamoto CA. Expression of the Candida albicans morphogenesis regulator gene CZF1 and its regulation by Efg1p and Czf1p. Eukaryot Cell 2006; 5: 825-835.
[29] Sato T, Watanabe T, Mikami T and Matsumoto T. Farnesol, a morphogenetic autoregulatory substance in the dimorphic fungus Candida albicans, inhibits hyphae growth through suppression of a mitogen-activated protein kinase cascade. Biol Pharm Bull 2004; 27: 751-752.
[30] Kruppa M, Krom BP, Chauhan N, Bambach AV, Cihlar RL and Calderone RA. The two-component signal transduction protein Chk1p regulates quorum sensing in Candida albicans. Eukaryot Cell 2004; 3: 1062-1065.
[31] Zakikhany K, Naglik JR, Schmidt-Westhausen A, Holland G, Schaller M and Hube B. In vivo transcript profiling of Candida albicans identifies a gene essential for interepithelial dissemination. Cell Microbiol 2007; 9: 2938-2954.
[32] Zeidler U, Lettner T, Lassnig C, Muller M, Lajko R, Hintner H, Breitenbach M and Bito A. UME6 is a crucial downstream target of other transcriptional regulators of true hyphal development in Candida albicans. FEMS Yeast Res 2009; 9: 126-142.
[33] Abe S, Tsunashima R, Iijima R, Yamada T, Maruyama N, Hisajima T, Abe Y, Oshima H and Yamazaki M. Suppression of anti-Candida activity of macrophages by a quorum-sensing molecule, farnesol, through induction of oxidative stress. Microbiol Immunol 2009; 53: 323-330.
[34] Hargarten JC, Moore TC, Petro TM, Nickerson KW and Atkin AL. Candida albicans quorum sensing molecules stimulate mouse macrophage migration. Infect Immun 2015; 83: 3857-3864.
[35] Leonhardt I, Spielberg S, Weber M, Albrecht-Eckardt D, Blass M, Claus R, Barz D, Scherlach K, Hertweck C, Loffler J, Hunniger K and Kurzai O. The fungal quorum-sensing molecule farnesol activates innate immune cells but suppresses cellular adaptive immunity. mBio 2015; 6: e00143.
[36] Zawrotniak M, Wojtalik K and Rapala-Kozik M. Farnesol, a quorum-sensing molecule of Candida albicans triggers the release of neutrophil extracellular traps. Cells 2019; 8: 1611.
[37] Hisajima T, Maruyama N, Tanabe Y, Ishibashi H, Yamada T, Makimura K, Nishiyama Y, Funa-

koshi K, Oshima H and Abe S. Protective effects of farnesol against oral candidiasis in mice. Microbiol Immunol 2008; 52: 327-333.
[38] Vivas W, Leonhardt I, Hunniger K, Hader A, Marolda A and Kurzai O. Multiple signaling pathways involved in human dendritic cell maturation are affected by the fungal quorum-sensing molecule farnesol. J Immunol 2019; 203: 2959-2969.
[39] Cottier F, Sherrington S, Cockerill S, Del Olmo Toledo V, Kissane S, Tournu H, Orsini L, Palmer GE, Perez JC and Hall RA. Remasking of Candida albicans beta-glucan in response to environmental pH is regulated by quorum sensing. mBio 2019; 10: e02347-19.
[40] Fang Y, Wu C, Wang Q and Tang J. Farnesol contributes to intestinal epithelial barrier function by enhancing tight junctions via the JAK/STAT3 signaling pathway in differentiated Caco-2 cells. J Bioenerg Biomembr 2019; 51: 403-412.
[41] Dizova S and Bujdakova H. Properties and role of the quorum sensing molecule farnesol in relation to the yeast Candida albicans. Pharmazie 2017; 72: 307-312.
[42] Kim D, Sengupta A, Niepa TH, Lee BH, Weljie A, Freitas-Blanco VS, Murata RM, Stebe KJ, Lee D and Koo H. Candida albicans stimulates Streptococcus mutans microcolony development via cross-kingdom biofilm-derived metabolites. Sci Rep 2017; 7: 41332.
[43] Zhu J, Krom BP, Sanglard D, Intapa C, Dawson CC, Peters BM, Shirtliff ME and Jabra-Rizk MA. Farnesol-induced apoptosis in Candida albicans is mediated by Cdr1-p extrusion and depletion of intracellular glutathione. PLoS One 2011; 6: e28830.
[44] Leger T, Garcia C and Camadro JM. The metacaspase (Mca1p) restricts O-glycosylation during farnesol-induced apoptosis in Candida albicans. Mol Cell Proteomics 2016; 15: 2308-2323.
[45] Jeong JH, Lee SE and Kim J. Mutational analysis of metacaspase CaMca1 and decapping activator Edc3 in the pathogenicity of Candida albicans. Fungal Genet Biol 2016; 97: 18-23.
[46] Singkum P, Muangkaew W, Suwanmanee S, Pumeesat P, Wongsuk T and Luplertlop N. Suppression of the pathogenicity of Candida albicans by the quorum-sensing molecules farnesol and tryptophol. J Gen Appl Microbiol 2020; 65: 277-283.
[47] Jung YY, Hwang ST, Sethi G, Fan L, Arfuso F and Ahn KS. Potential anti-inflammatory and anticancer properties of farnesol. Molecules 2018; 23: 2827.
[48] Lee JH, Kim C, Kim SH, Sethi G and Ahn KS. Farnesol inhibits tumor growth and enhances the anticancer effects of bortezomib in multiple myeloma xenograft mouse model through the modulation of STAT3 signaling pathway. Cancer Lett 2015; 360: 280-293.
[49] Delmondes GA, Santiago Lemos IC, Dias DQ, Cunha GLD, Araújo IM, Barbosa R, Coutinho HDM, Felipe CFB, Barbosa-Filho JM, Lima NTR, De Menezes IRA and Kerntopf MR. Pharmacological applications of farnesol (C15H26O): a patent review. Expert Opin Ther Pat 2020; 30: 227-234.
[50] Todd OA and Peters BM. Candida albicans and Staphylococcus aureus pathogenicity and polymicrobial interactions: lessons beyond Koch's Postulates. J Fungi (Basel) 2019; 5: 81.
[51] Kong EF, Tsui C, Kucharikova S, Van Dijck P and Jabra-Rizk MA. Modulation of Staphylococcus aureus response to antimicrobials by the Candida albicans quorum sensing molecule farnesol. Antimicrob Agents Chemother 2017; 61: e01573-17.
[52] Vila T, Kong EF, Ibrahim A, Piepenbrink K, Shetty AC, McCracken C, Bruno V and Jabra-Rizk MA. Candida albicans quorum-sensing molecule farnesol modulates staphyloxanthin production and activates the thiol-based oxidative-stress response in Staphylococcus aureus. Virulence 2019; 10: 625-642.
[53] Inoue Y, Togashi N and Hamashima H. Farnesol-induced disruption of the Staphylococcus aureus cytoplasmic membrane. Biol Pharm Bull 2016; 39: 653-656.
[54] Constantino JA, Delgado-Rastrollo M, Pacha-Olivenza MA, Perez-Giraldo C, Quiles M, Gonzalez-Martin ML and Gallardo-Moreno AM. In vivo bactericidal efficacy of farnesol on Ti6Al4V implants. Rev Esp Cir Ortop Traumatol 2016; 60: 260-266.
[55] Cao L, Zhang ZZ, Xu SB, Ma M and Wei X. Farnesol inhibits development of caries by augmenting oxygen sensitivity and suppressing virulence-associated gene expression in Staphylococcus mutans. J Biomed Res 2017; 31: 333-343.
[56] Fernandes RA, Monteiro DR, Arias LS, Fernandes GL, Delbem AC and Barbosa DB. Biofilm formation by Candida albicans and Streptococcus mutans in the presence of farnesol: a quantitative evaluation. Biofouling 2016; 32: 329-338.
[57] Fernandes RA, Monteiro DR, Arias LS, Fernandes GL, Delbem ACB and Barbosa DB. Virulence factors in Candida albicans and Streptococcus mutans biofilms mediated by farnesol. Indian J Microbiol 2018; 58: 138-145.
[58] Rocha GR, Florez Salamanca EJ, de Barros AL, Lobo CIV and Klein MI. Effect of tt-farnesol and myricetin on in vitro biofilm formed by Streptococcus mutans and Candida albicans. BMC Complement Altern Med 2018; 18: 61.

[59] Cernakova L, Jordao L and Bujdakova H. Impact of farnesol and Corsodyl® on Candida albicans forming dual biofilm with Streptococcus mutans. Oral Dis 2018; 24: 1126-1131.
[60] Egbe NE, Dornelles TO, Paget CM, Castelli LM and Ashe MP. Farnesol inhibits translation to limit growth and filamentation in C. albicans and S. cerevisiae. Microb Cell 2017; 4: 294-304.
[61] Kischkel B, Souza GK, Chiavelli LUR, Pomini AM, Svidzinski TIE and Negri M. The ability of farnesol to prevent adhesion and disrupt Fusarium keratoplasticum biofilm. Appl Microbiol Biotechnol 2020; 104: 377-389.
[62] Bahn YS and Sundstrom P. CAP1, an adenylate cyclase-associated protein gene, regulates bud-hypha transitions, filamentous growth, and cyclic AMP levels and is required for virulence of Candida albicans. J Bacteriol 2001; 183: 3211-3223.
[63] Cheetham J, Smith DA, da Silva Dantas A, Doris KS, Patterson MJ, Bruce CR and Quinn J. A single MAPKKK regulates the Hog1 MAPK pathway in the pathogenic fungus Candida albicans. Mol Biol Cell 2007; 18: 4603-4614.
[64] Singh S, Rehman S, Fatima Z and Hameed S. Protein kinases as potential anticandidal drug targets. Front Biosci (Landmark Ed) 2020; 25: 1412-1432.
[65] Yamada-Okabe T, Mio T, Ono N, Kashima Y, Matsui M, Arisawa M and Yamada-Okabe H. Roles of three histidine kinase genes in hyphal development and virulence of the pathogenic fungus Candida albicans. J Bacteriol 1999; 181: 7243-7247.
[66] Calera JA, Zhao XJ and Calderone R. Defective hyphal development and avirulence caused by a deletion of the SSK1 response regulator gene in Candida albicans. Infect Immun 2000; 68: 518-525.
[67] Herrero-de-Dios C, Alonso-Monge R and Pla J. The lack of upstream elements of the Cek1 and Hog1 mediated pathways leads to a synthetic lethal phenotype upon osmotic stress in Candida albicans. Fungal Genet Biol 2014; 69: 31-42.
[68] Su C, Lu Y and Liu H. Reduced TOR signaling sustains hyphal development in Candida albicans by lowering Hog1 basal activity. Mol Biol Cell 2013; 24: 385-397.
[69] Urrialde V, Prieto D, Hidalgo-Vico S, Roman E, Pla J and Alonso-Monge R. Deletion of the SKO1 gene in a hog1 mutant reverts virulence in Candida albicans. J Fungi (Basel) 2019; 5: 107.
[70] Loewith R and Hall MN. Target of rapamycin (TOR) in nutrient signaling and growth control. Genetics 2011; 189: 1177-1201.
[71] Lu Y, Su C and Liu H. A GATA transcription factor recruits Hda1 in response to reduced Tor1 signaling to establish a hyphal chromatin state in Candida albicans. PLoS Pathog 2012; 8: e1002663.
[72] Zacchi LF, Gomez-Raja J and Davis DA. Mds3 regulates morphogenesis in Candida albicans through the TOR pathway. Mol Cell Biol 2010; 30: 3695-3710.
[73] Roman E, Nombela C and Pla J. The Sho1 adaptor protein links oxidative stress to morphogenesis and cell wall biosynthesis in the fungal pathogen Candida albicans. Mol Cell Biol 2005; 25: 10611-10627.
[74] Roman E, Cottier F, Ernst JF and Pla J. Msb2 signaling mucin controls activation of Cek1 mitogen-activated protein kinase in Candida albicans. Eukaryot Cell 2009; 8: 1235-1249.
[75] Csank C, Schroppel K, Leberer E, Harcus D, Mohamed O, Meloche S, Thomas DY and Whiteway M. Roles of the Candida albicans mitogen-activated protein kinase homolog, Cek1p, in hyphal development and systemic candidiasis. Infect Immun 1998; 66: 2713-2721.
[76] Eisman B, Alonso-Monge R, Roman E, Arana D, Nombela C and Pla J. The Cek1 and Hog1 mitogen-activated protein kinases play complementary roles in cell wall biogenesis and chlamydospore formation in the fungal pathogen Candida albicans. Eukaryot Cell 2006; 5: 347-358.
[77] Yang T, Li W, Li Y, Liu X and Yang D. The ESCRT System Plays an Important Role in the Germination in Candida albicans by regulating the expression of hyphal-specific genes and the localization of polarity-related proteins. Mycopathologia 2020; 185: 439-454.
[78] Mu C, Pan C, Han Q, Liu Q, Wang Y and Sang J. Phosphatidate phosphatase Pah1 has a role in the hyphal growth and virulence of Candida albicans. Fungal Genet Biol 2019; 124: 47-58.
[79] Nikoomanesh F, Roudbarmohammadi S, Khoobi M, Haghighi F and Roudbary M. Design and synthesis of mucoadhesive nanogel containing farnesol: investigation of the effect on HWP1, SAP6 and Rim101 genes expression of Candida albicans in vitro. Artif Cells Nanomed Biotechnol 2019; 47: 64-72.
[80] Ceresa C, Tessarolo F, Maniglio D, Caola I, Nollo G, Rinaldi M and Fracchia L. Inhibition of Candida albicans biofilm by lipopeptide AC7 coated medical-grade silicone in combination with farnesol. Aims Bioengineering 2018; 5: 192-208.
[81] Bozo A, Doman M, Majoros L, Kardos G, Varga I and Kovacs R. The in vitro and in vivo efficacy of fluconazole in combination with farnesol against Candida albicans isolates using a mu-

rine vulvovaginitis model. J Microbiol 2016; 54: 753-760.
[82] Cernakova L, Dizova S, Gaskova D, Jancikova I and Bujdakova H. Impact of farnesol as a modulator of efflux pumps in a fluconazole-resistant strain of Candida albicans. Microb Drug Resist 2019; 25: 805-812.
[83] Liu Z, Rossi JM and Myers LC. Candida albicans Zn cluster transcription factors Tac1 and Znc1 are activated by farnesol to upregulate a transcriptional program including the multidrug efflux pump CDR1. Antimicrob Agents Chemother 2018; 62: e00968-18.
[84] Dizova S, Cernakova L and Bujdakova H. The impact of farnesol in combination with fluconazole on Candida albicans biofilm: regulation of ERG20, ERG9, and ERG11 genes. Folia Microbiol (Praha) 2018; 63: 363-371.
[85] Xia J, Qian F, Xu W, Zhang Z and Wei X. In vitro inhibitory effects of farnesol and interactions between farnesol and antifungals against biofilms of Candida albicans resistant strains. Biofouling 2017; 33: 283-293.
[86] Touil HFZ, Boucherit K, Boucherit-Otmani Z, Kohder G, Madkour M and Soliman SSM. Optimum inhibition of amphotericin-B-resistant Candida albicans strain in single- and mixed-species biofilms by Candida and non-Candida terpenoids. Biomolecules 2020; 10: 342.
[87] Monteiro DR, Arias LS, Fernandes RA, Deszo da Silva LF, de Castilho M, da Rosa TO, Vieira APM, Straioto FG, Barbosa DB and Delbem ACB. Antifungal activity of tyrosol and farnesol used in combination against Candida species in the planktonic state or forming biofilms. J Appl Microbiol 2017; 123: 392-400.
[88] Sebaa S, Boucherit-Otmani Z and Courtois P. Effects of tyrosol and farnesol on Candida albicans biofilm. Mol Med Rep 2019; 19: 3201-3209.
[89] Arias LS, Delbem AC, Fernandes RA, Barbosa DB and Monteiro DR. Activity of tyrosol against single and mixed-species oral biofilms. J Appl Microbiol 2016; 120: 1240-1249.
[90] Barot T, Rawtani D, Kulkarni P, Hussain CM and Akkireddy S. Physicochemical and biological assessment of flowable resin composites incorporated with farnesol loaded halloysite nanotubes for dental applications. J Mech Behav Biomed Mater 2020; 104: 103675.
[91] Bandara HM, Herpin MJ, Kolacny D Jr, Harb A, Romanovicz D and Smyth HD. Incorporation of farnesol significantly increases the efficacy of liposomal ciprofloxacin against Pseudomonas aeruginosa biofilms in vitro. Mol Pharm 2016; 13: 2760-2770.
[92] Li Y, Shan M, Yan M, Yao H, Wang Y, Gu B, Zhu Z and Li H. Anticandidal activity of kalopanaxsaponin A: effect on proliferation, cell morphology, and key virulence attributes of Candida albicans. Front Microbiol 2019; 10: 2844.
[93] Ying L, Mingzhu S, Mingju Y, Ye X, Yuechen W, Ying C, Bing G, Hongchun L and Zuobin Z. The inhibition of trans-cinnamaldehyde on the virulence of Candida albicans via enhancing farnesol secretion with low potential for the development of resistance. Biochem Biophys Res Commun 2019; 515: 544-550.
[94] Liu RH, Shang ZC, Li TX, Yang MH and Kong LY. In vitro antibiofilm activity of eucarobustol E against Candida albicans. Antimicrob Agents Chemother 2017; 61: e02707-16.
[95] Zhang M, Chang W, Shi H, Zhou Y, Zheng S, Li Y, Li L and Lou H. Biatriosporin D displays antivirulence activity through decreasing the intracellular cAMP levels. Toxicol Appl Pharmacol 2017; 322: 104-112.
[96] Yan Y, Tan F, Miao H, Wang H and Cao Y. Effect of shikonin against Candida albicans biofilms. Front Microbiol 2019; 10: 1085.
[97] Shin DS and Eom YB. Zerumbone inhibits Candida albicans biofilm formation and hyphal growth. Can J Microbiol 2019; 65: 713-721.
[98] Shin DS and Eom YB. Efficacy of zerumbone against dual-species biofilms of Candida albicans and Staphylococcus aureus. Microb Pathog 2019; 137: 103768.