Title

The Als3 Cell Wall Adhesin Plays a Critical Role in Human Serum Amyloid A1-Induced Cell Death and Aggregation in Candida albicans.

Journal

Antimicrobial Agents and Chemotherapy, 64(6)

ISSN

0066-4804

Authors

Gong, Jiao
Bing, Jian
Guan, Guobo
et al.

Publication Date

2020-05-21

DOI

10.1128/aac.00024-20

Peer reviewed

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

MECHANISMS OF RESISTANCE

The Als3 Cell Wall Adhesin Plays a Critical Role in Human Serum Amyloid A1-Induced Cell Death and Aggregation in *Candida albicans*

Jiao Gong,[a,b] Jian Bing,[a] Guobo Guan,[b] Clarissa J. Nobile,[c] Guanghua Huang[a]

[a]Department of Infectious Diseases, Huashan Hospital and State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Shanghai, China
[b]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China
[c]Department of Molecular and Cell Biology, School of Natural Sciences, University of California, Merced, Merced, California, USA

ABSTRACT Antimicrobial peptides and proteins play critical roles in the host defense against invading pathogens. We recently discovered that recombinantly expressed human and mouse serum amyloid A1 (rhSAA1 and rmSAA1, respectively) proteins have potent antifungal activities against the major human fungal pathogen *Candida albicans*. At high concentrations, rhSAA1 disrupts *C. albicans* membrane integrity and induces rapid fungal cell death. In the present study, we find that rhSAA1 promotes cell aggregation and targets the *C. albicans* cell wall adhesin Als3. Inactivation of *ALS3* in *C. albicans* leads to a striking decrease in cell aggregation and cell death upon rhSAA1 treatment, suggesting that Als3 plays a critical role in SAA1 sensing. We further demonstrate that deletion of the transcriptional regulators controlling the expression of *ALS3*, such as *AHR1*, *BCR1*, and *EFG1*, in *C. albicans* results in similar effects to that of the *als3/als3* mutant upon rhSAA1 treatment. Global gene expression profiling indicates that rhSAA1 has a discernible impact on the expression of cell wall- and metabolism-related genes, suggesting that rhSAA1 treatment could lead to a nutrient starvation effect on *C. albicans* cells.

KEYWORDS *Candida albicans*, SAA1, adhesins, Als3, antifungal activity, cell aggregation, serum amyloid A

Candida albicans is a commensal of mucosal surfaces and is also the most prevalent fungal pathogen of humans. It causes both superficial diseases such as oral thrush and vaginitis and life-threatening disseminated infections (1). The interplay between the host innate immune system and *C. albicans* represents a good example of an evolutionary arms race (2). The host can produce a set of antimicrobial peptides and proteins (AMPs) to clear invading pathogens, while pathogens devise strategies to evade these host defenses. *C. albicans*, for example, has developed a variety of evasion strategies, including morphological transitions, which it uses to rapidly adapt to the host environment and to evade the host innate immune system.

Mammalian serum amyloid A (SAA) proteins are a conserved family of apolipoproteins associated with high-density lipoprotein (HDL) in plasma (3). In response to inflammatory stimuli, some members of this family (e.g., SAA1) are induced in hepatocytes. The protein level of SAA1 in plasma can increase >1,000-fold during an acute-phase response (3). It has been reported that SAA1 has potent antibacterial activity (3–5). Recombinant human SAA1 is able to form ion channels in planar lipid bilayer membranes (4) and has also been found to target the outer membranes of bacteria (6). Mouse intestinal epithelial and hepatic SAAs can act as modulators in the control of bacterial growth (5). In a recent study, we demonstrated that recombinant human and mouse SAA1 proteins (rhSAA1 and rmSAA1, respectively) exhibit potent

Citation Gong J, Bing J, Guan G, Nobile CJ, Huang G. 2020. The Als3 cell wall adhesin plays a critical role in human serum amyloid A1-induced cell death and aggregation in *Candida albicans*. Antimicrob Agents Chemother 64:e00024-20. https://doi.org/10.1128/AAC.00024-20.

Address correspondence to Guanghua Huang, huanggh@im.ac.cn.

Received 6 January 2020
Returned for modification 26 February 2020
Accepted 16 March 2020
Accepted manuscript posted online 23 March 2020
Published 21 May 2020

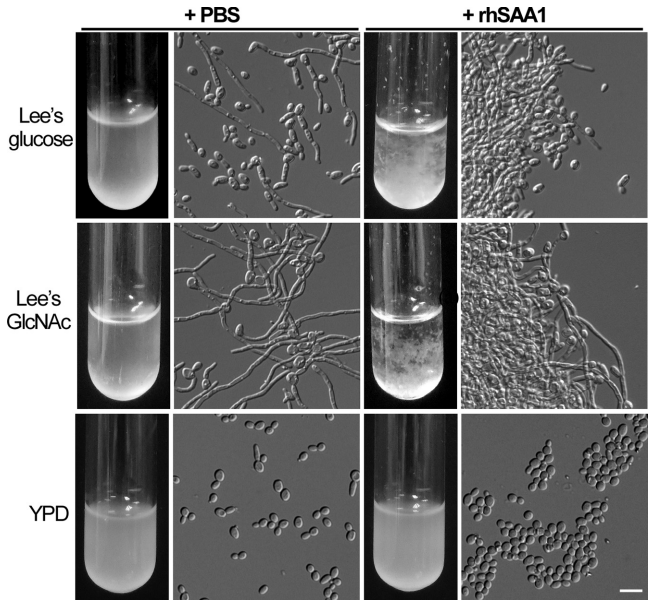

FIG 1 rhSAA1 induces cell aggregation in *C. albicans*. *C. albicans* cells (SC5314) were cultured to mid-exponential phase in liquid Lee's glucose, Lee's GlcNAc, and YPD media at 30°C with shaking. Fungal cells (2 ml) were then treated with rhSAA1 (at a final concentration of 40 mg/liter) for 1 h at 30°C with shaking at 200 rpm. The cultures were then gently shaken before being photographed. PBS treatment served as a negative control. Bar, 10 μm.

antifungal activities against *C. albicans* (7). rhSAA1 targets the cell surface of *C. albicans* cells and impairs the integrity of the fungal cell membrane. However, the molecular mechanisms through which SAA1 exerts its effects on this fungus remain largely unknown.

In the present study, we report that treatment with rhSAA1 leads to a global change in gene expression and induces rapid cell aggregation in *C. albicans*. Mechanistic investigation into the function of rhSAA1 suggests that rhSAA1 targets Als3 (agglutinin-like sequence 3), a cell wall adhesin of *C. albicans*. Inactivation of *C. albicans* Als3 results in a reduced susceptibility to rhSAA1-induced cell death and aggregation.

RESULTS

rhSAA1 induces cell aggregation in *C. albicans*. The ability to sense and rapidly respond to attack by the host immune system is critical for fungal pathogens to survive in the host. In our killing assays, we observed that *C. albicans* cells rapidly aggregated upon treatment with rhSAA1 (7). To verify this phenomenon, we treated *C. albicans* cells with rhSAA1 and performed cell aggregation assays in three different media: yeast extract-peptone-dextrose (YPD), Lee's glucose, and Lee's GlcNAc. As shown in Fig. 1, *C. albicans* cells filamented and formed aggregates (or "flocs") in Lee's glucose and Lee's GlcNAc media. In YPD medium, *C. albicans* cells also aggregated, although cells maintained the yeast form.

SAA proteins are able to undergo autoaggregation and form amyloid fibrils at certain threshold concentrations (8). We predicted that rhSAA1 induces cell aggregation in *C. albicans* through two possible mechanisms. One possibility is that the intercellular interaction and autoaggregation of rhSAA1 binding to the fungal cell surface could by consequence induce *C. albicans* aggregation. The second possibility is that rhSAA1 activates the endogenous signaling pathway that is responsible for fungal cell aggregation. To determine the mechanism of *C. albicans* cell aggregation, we treated both live and heat-killed *C. albicans* cells with rhSAA1. As shown in Fig. 2, rhSAA1 treatment caused cell aggregation in live cells but not in heat-killed cells of *C. albicans*. Since cell viability is essential for this response, these results suggest that the aggregation of *C. albicans* cells is due to the activation of the fungal endogenous signaling pathway upon rhSAA1 treatment.

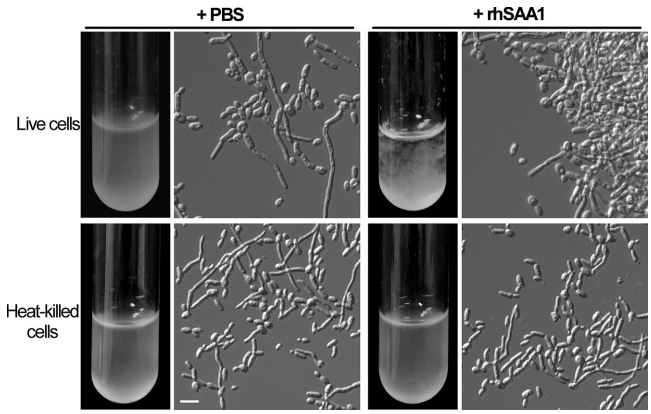

FIG 2 rhSAA1 does not induce aggregation in heat-killed *C. albicans* cells. *C. albicans* cells (SC5314) were cultured to mid-exponential phase in liquid Lee's glucose at 30°C. To induce cell killing, *C. albicans* cells were incubated at 100°C for 10 min. Live or heat-killed cells (2 ml) were then treated with rhSAA1 (at a final concentration of 40 mg/liter) for 1 h at 30°C with shaking. The cultures were then gently shaken before being photographed. PBS treatment served as a negative control. Bar, 10 μm.

Global transcriptional effects of rhSAA1 on *C. albicans*. To further explore the functional mechanism of rhSAA1-induced cell death and aggregation in *C. albicans*, we performed RNA sequencing (RNA-Seq) to compare the transcriptional profiles of rhSAA1-treated and phosphate-buffered saline (PBS)-treated (control) cells of *C. albicans*. We found 491 differentially expressed genes (using a 2-fold change cutoff). Of them, 162 genes were downregulated and 329 genes were upregulated in rhSAA1-treated cells (Fig. 3a; see also Data Set S1 in the supplemental material). Upon rhSAA1 treatment, two striking features of gene expression were observed.

First, metabolism-associated genes, including those involved in glycolysis and mitochondrial and energy metabolism, were upregulated. Moreover, genes involved in ion metabolism exhibited a notable change when treated with rhSAA1. For example, iron utilization-associated genes (*FET34*, *FTR1*, *SFU1*, and *CFL2*) were upregulated, whereas *FRE7* and *FRE30* were downregulated upon rhSAA1 treatment. In addition, several copper-related genes, such as *CRP1*, *COX17*, and *SCO1* were also upregulated upon rhSAA1 treatment. This induced starvation response indicates that the binding of rhSAA1 to the fungal cell somehow functions as a signal for nutrient utilization.

Second, several cell wall-related genes, including cell wall protein-encoding genes and cell wall maintenance regulators, were downregulated, while many genes involved in cell membrane maintenance were upregulated (Fig. 3b and Data Set S1). For example, cell wall structure-related genes, such as *CHT4* (encoding a chitinase), *SKN1* and *KRE5* (required for β-1,6-glucan synthesis), *ALG5* (encoding a glucosyltransferase), and *BMT1* and *BMT3* (encoding β-mannosyltransferases) were significantly downregulated after treatment with 40 mg/liter rhSAA1. Genes involved in cell wall regeneration and cell wall integrity (*PMT1*, *PMT2*, and *AMS1*) were also downregulated after treatment with rhSAA1. Ergosterol is an important component of the fungal cell membrane. Enzymes of the ergosterol biosynthetic pathway are the most common targets of clinically used antifungal drugs. We found that a number of genes involved in the synthesis of ergosterol (*ERG3*, *ERG11*, *ERG251*, and *UPC2*) were upregulated upon treatment with rhSAA1. Two sphingolipid metabolism-related genes, *SLD1* and *CRG1*, were also upregulated. To validate the RNA-Seq data, 22 representative differentially expressed genes were selected and examined for relative expression levels in response to treatment with rhSAA1. As shown in Fig. S1, the relative expression levels were generally consistent with the results of the RNA-Seq data. Taken together, the alterations in cell wall-related and membrane-related genes upon rhSAA1 treatment may represent a stress response to the damaging effects of rhSAA1 on the cell membrane of *C. albicans*. These results are consistent with the disruptive effects of SAA1 on fungal cell membrane integrity (7).

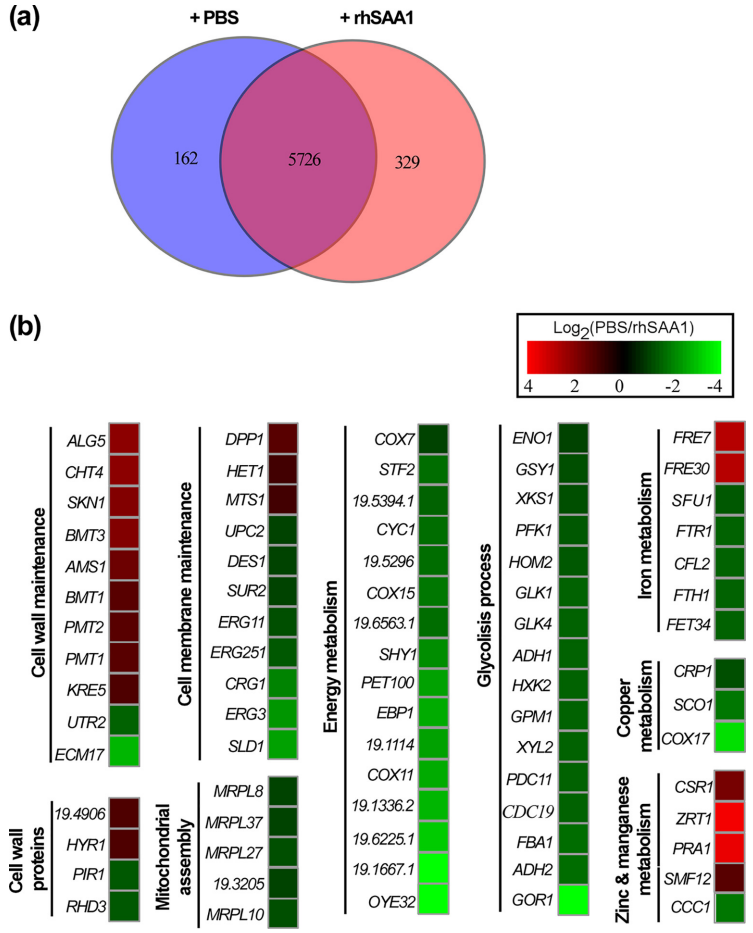

FIG 3 Effect of rhSAA1 treatment on the global transcriptional expression profile of *C. albicans*. (a) Gene expression Venn diagram. A 2-fold difference cutoff, false discovery rates (FDRs) of less than 0.05, and fragment per kilobase of transcript per million (FPKM) of at least one sample more than 20 were used to define differentially expressed genes. (b) Heatmap of selected differentially expressed genes generated with R package software. Functional categories of genes are indicated. Log_2(PBS/rhSAA1), Log_2(read count of PBS-treated sample/read count of rhSAA1-treated sample). A detailed analysis is presented in Data Set S1 in the supplemental material.

rhSAA1 targets the cell wall protein Als3 in *C. albicans*. Since rhSAA1 induces cell aggregation and binds to the cell surface of *C. albicans*, we predicted that rhSAA1 could target specific *C. albicans* cell wall proteins. To identify the rhSAA1 targets, we tested the ability of a subset of *C. albicans* cell wall protein mutants from our collections and the mutant library generated by the Noble lab (9) for cell aggregation in the presence of rhSAA1. In this screen, we identified that the mutant of *ALS3* but not *ALS4* exhibited a defect in cell aggregation upon rhSAA1 treatment (Fig. 4a). *ALS3* and *ALS4* encode two glycosylphosphatidylinositol (GPI)-anchored proteins belonging to the Als family (10). Als3 is a known cell wall adhesin that is required for epithelial adhesion and endothelial invasion (10–12). As expected, cells of the wild-type (WT) and *ALS3*-reconstituted strains had similar levels of aggregation when treated with rhSAA1. Consistent with this result, cells of the *als3/als3* mutant demonstrated a significantly higher survival rate than those of the WT and *ALS3*-reconstituted strains in killing assays (Fig. 4b), suggesting that Als3 is a potential target of rhSAA1 and plays a critical role in rhSAA1-induced cell death and cell aggregation.

To determine whether there is a physical interaction between Als3 and rhSAA1, we ectopically expressed *C. albicans ALS3* in *Saccharomyces cerevisiae* whose genome lacks *ALS* homologs. Immunofluorescence assays illustrated that rhSAA1 could bind to *S. cerevisiae* cells expressing *C. albicans ALS3* but not to control cells containing an empty

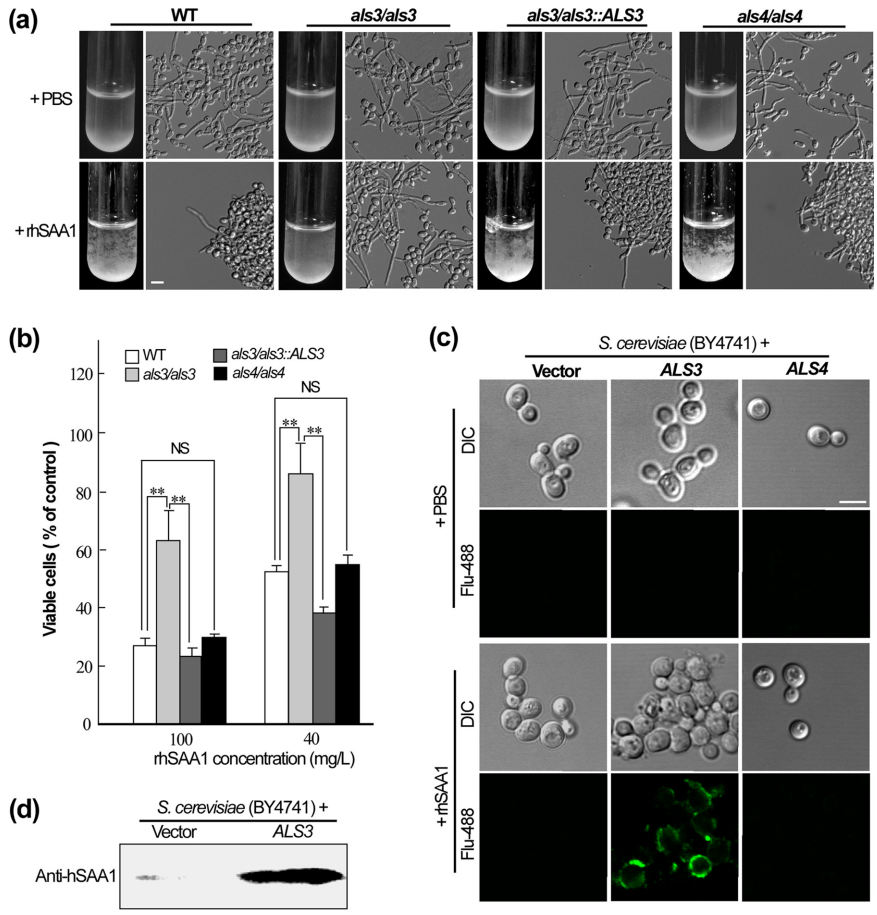

FIG 4 Distinct roles of Als3 and Als4 in rhSAA1-induced cell death and aggregation in *C. albicans*. (a) Cell aggregation in the WT (SN152**a**), *als3/als3*, *ALS3*-reconstituted, and *als4/als4* strains. *C. albicans* cells were cultured to the mid-exponential phase in liquid Lee's glucose medium at 30°C with shaking. Fungal cells (2 ml) were then treated with rhSAA1 (at a final concentration of 40 mg/liter) for 1 h at 30°C with shaking at 200 rpm. The cultures were then gently shaken before being photographed. PBS treatment served as a negative control. Bar, 10 μm. (b) Susceptibility of the WT(SN152**a**), *als3/als3*, *ALS3*-reconstituted, and *als4/als4* strains to rhSAA1. *C. albicans* cells (2×10^5 cells/ml) of the WT, *als3/als3*, *als4/als4*, and *ALS3*-reconstituted strains were treated with 40 mg/liter or 100 mg/liter rhSAA1 for 3 h in Lee's glucose at 30°C. PBS treatment served as a negative control. The ratio of viable cells was determined using plating assays. The percentage of viable cells = (number of colonies of the rhSAA1-treated sample/average number of colonies of the PBS-treated sample) $\times$ 100. Values are presented as means $\pm$ standard deviations (SDs) ($n = 3$). **, $P < 0.01$; NS, no significance (Student's *t* test, two-tailed). (c) rhSAA1 binds to the cell surface of *CaALS3*-heterologous expression cells of *S. cerevisiae* (BY4741). *S. cerevisiae* cells heterologously expressing *C. albicans ALS3* or *ALS4* were cultured to the mid-exponential phase in liquid YPD medium at 30°C, collected, and washed with PBS. Cells (5×10^6 cells/ml) were then treated with 200 mg/liter rhSAA1 or PBS for 2.5 h at room temperature (RT). Anti-hSAA1 antibody and Alexa Fluor 488-conjugated anti-mouse IgG were used for immunofluorescence staining assays. Control, BY4741 plus vector. (d) Immunoblot assay; 1×10^7 cells of the control and *CaALS3*-heterologously expressing cells of *S. cerevisiae* were treated with 200 mg/liter rhSAA1 for 2.5 h at RT. Cells were collected and eluted with 6 M urea. The binding of rhSAA1 to *S. cerevisiae* cells was then detected by Western blotting.

vector or cells expressing *C. albicans ALS4* (Fig. 4c). We then collected the control cells and *S. cerevisiae* cells expressing *C. albicans ALS3* treated with rhSAA1 and performed Western blot assays. Consistent with the results of the immunofluorescence assays, a strong hybrid signal of rhSAA1 was detected in the sample of *S. cerevisiae* cells expressing *C. albicans ALS3* but was not in the control sample (Fig. 4d). Taken together, these results suggest that rhSAA1 functions by directly binding to the *C. albicans* cell wall protein Als3.

Ahr1, Bcr1, and Efg1 are required for rhSAA1-induced cell death and aggregation in *C. albicans*. Ahr1, Bcr1, and Efg1 are important transcription factors involved in the regulation of cell wall proteins and adhesion in *C. albicans* (13, 14). Inactivation

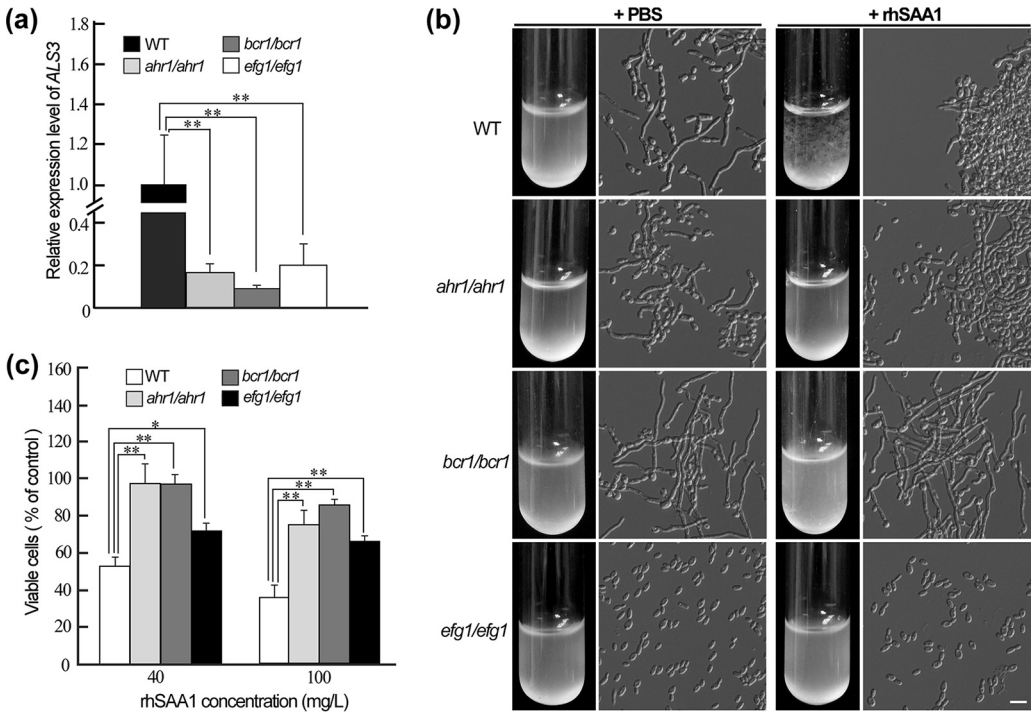

FIG 5 Killing of *C. albicans* cells of transcription factor mutants by rhSAA1. (a) Relative expression level of *ALS3* in the WT (SN250A-), *ahr1/ahr1*, *bcr1/bcr1*, and *efg1/efg1* mutants. Total RNA was extracted from *C. albicans* cells grown in liquid Lee's glucose medium at 30°C and used for quantitative RT-PCR assays. The relative expression level of *ALS3* in WT was set as "1." Error bar, standard deviation. **, $P < 0.01$ (Student's *t* test, two-tailed). (b) Effect of rhSAA1 treatment on cell aggregation of the WT (SN250A-), *ahr1/ahr1*, *bcr1/bcr1*, and *efg1/efg1* mutants. Two milliliters of *C. albicans* cells cultured in liquid Lee's glucose medium was treated with rhSAA1 (at a final concentration of 40 mg/liter) for 1 h at 30°C with shaking at 200 rpm. The sample tubes were gently shaken before imaging. PBS treatment served as a negative control. (c) Killing effect of rhSAA1 on the WT (SN250A-), *ahr1/ahr1*, *bcr1/bcr1*, and *efg1/efg1* mutants; 2×10^5 cells/ml *C. albicans* cells were incubated with 40 mg/liter or 100 mg/liter rhSAA1 for 3 h in Lee's glucose at 30°C. PBS treatment served as a negative control. Viable cells were determined using plating assays. The percentage of viable cells = (number of colonies of the rhSAA1-treated sample/average number of colonies of the PBS-treated sample) × 100. Values are means ± SDs ($n = 3$). *, $P < 0.05$; **, $P < 0.01$ (Student's *t* test, two-tailed).

of *EFG1*, *BCR1*, or *AHR1* remarkably reduced the transcriptional expression of *ALS3* in *C. albicans* (Fig. 5a). Given the key role of Als3 in rhSAA1-mediated cell death in *C. albicans*, we predicted that deletion of these transcriptional regulators would lead to similar responses to rhSAA1 as deletion of *ALS3*. Indeed, killing and cell aggregation assays demonstrated that cells of the *ahr1/ahr1*, *bcr1/bcr1*, and *efg1/efg1* mutants exhibited similar response patterns to the treatment of rhSAA1 to those of the *als3/als3* mutant (Fig. 5b and c). These findings confirm the key role of Als3 in rhSAA1-mediated cell death and flocculation.

DISCUSSION

The interactions of *C. albicans* with the host are critical for its commensal and pathogenic life styles. Fungal pathogens often express numerous adhesins, secreted aspartyl proteinases, and even fungal toxins, such as candidalysin, to facilitate invasion of the host. On the other hand, upon fungal infection, the host has a number of defense strategies, including secreting AMPs, "nutritional immunity," and producing antibodies through adaptive immunity to clear invading fungal pathogens (15). Mammalian SAA1 is a major acute-phase protein that has antimicrobial activity against both bacteria and fungi (5, 7). In the present study, we set out to investigate the antifungal mechanism of human SAA1 against *C. albicans*. We demonstrate that rhSAA1 induces cell aggregation in *C. albicans* and potentially targets the cell surface protein Als3. The Als3 adhesin is required for rhSAA1-induced cell death and aggregation in *C. albicans*.

We previously found that rhSAA1 binds to the cell surface of *C. albicans* and disrupts

membrane integrity (7). Interestingly, unlike most membrane-disrupting AMPs, human SAA1 is hydrophilic and negatively charged (16), and native SAA1 often exists as a hexamer. These structural features may favor its conformational changes and ability to bind to the fungal cell surface (8). Using genetic and biochemical assays, we identify *C. albicans* Als3 as a potential target of rhSAA1. It is possible that the Als3 adhesin could function as an anchor of host SAA1. After binding to Als3, SAA1 could form a hexamer and disrupt the cell membrane of *C. albicans*. Moreover, Als3 is a crucial hypha-specific cell wall adhesin of *C. albicans* and plays a critical role in host epithelial adhesion (17). Als3 is a species-specific adhesin only found in *C. albicans* and its closely related species *Candida dubliniensis*. Perhaps due to the lack of an ortholog of Als3 in *Candida glabrata*, *Candida parapsilosis*, and *S. cerevisiae*, rhSAA1 exhibited no killing effect on cells of these fungi (7). Interestingly, Als3 contains a conservative amyloid-forming region (AFR; 325 to 331 amino acids [aa]) that is required for the formation of amyloid fibers and its aggregative function (11). It remains to be investigated whether this AFR region is important for the Als3-rhSAA1 physical interaction and for host sensing of *C. albicans*.

From the pathogen perspective, SAA1-induced cell aggregation could be a *C. albicans* adaptive behavior to increase its survival upon host immune molecular attacks. Global transcriptional profiling demonstrated that rhSAA1 treatment led to a global change of gene expression in *C. albicans*. Strikingly, cell wall-related genes were generally downregulated, whereas cell membrane maintenance-related and metabolism-related genes were upregulated upon rhSAA1 treatment. For example, the expression of a large number of metabolism-related genes, including many oxidative metabolism- and tricarboxylic acid (TCA) cycle-related genes, were upregulated in rhSAA1-treated cells of *C. albicans* (Fig. 3 and Data Set S1 in the supplemental material). This increased expression of metabolism-related genes implies that the binding of rhSAA1 protein to the fungal surface affects the intake of nutritional components and results in an induced starvation. These changes in gene expression could facilitate the fungus to better survive in the host. Similar to SAA1, mammalian interleukin 17A (IL-17A) also induces cellular aggregation in *C. albicans* by targeting GPI-anchored cell wall proteins (18). Therefore, to adapt to the host environment and respond to host defense mechanisms, *C. albicans* has evolved elegant means to sense and respond to the host.

The innate immune response to invading pathogenic *Candida* species is the first line of host defense. Given the conserved characteristic of SAA1 in mammals, one would expect that the rapid increase of SAA1 plasma levels upon acute-phase response to infection would facilitate the rapid clearance of invading pathogens. During long-term coevolution, *C. albicans* has also developed a repertoire of protective strategies to survive host immune attacks. Our discovery of the potential target of human SAA1 in *C. albicans* may be useful in the development of novel antifungal strategies.

MATERIALS AND METHODS

Strains and growth conditions. The strains used in this study are listed in Table S1 in the supplemental material. YPD medium (2% glucose, 2% peptone, 1% yeast extract) was used for routine growth of *C. albicans* and *S. cerevisiae* strains. Modified Lee's glucose and Lee's GlcNAc media (pH 6.8) were used for cellular aggregation assays in *C. albicans*. LB medium (1% peptone, 1% NaCl, 0.5% yeast extract) was used for bacterial growth.

The two alleles of *ALS3* were deleted using a homologous recombination strategy with fusion PCR products in *C. albicans* as described previously (19). Briefly, the two alleles of *ALS3* were replaced with *ARG4* and *HIS1* selectable markers that were generated by fusion PCR strategies. The *ARG4* and *HIS1* cassettes were amplified from plasmids pRS-ARG4ΔSpeI and pGEM-HIS1, respectively (20). The *als4/als4* mutant was generated using a similar strategy. Primers used for PCRs are listed in Table S2.

To construct the *ALS3*-reconstituted strain, fusion PCR products containing *ALS3* and the *CaSAT1* cassette (nourseothricin-resistant marker adapted for *Candida* species) were generated and transformed into the *als3/als3* mutant of *C. albicans*. The *CaSAT1* cassette was amplified from plasmid pSF2A (21); fragments of 3′ untranslated region (UTR) of *ALS3* and *ALS3* open reading frame (ORF) plus 5′ UTR were amplified from genomic DNA of *C. albicans* SC5314. The *CaSAT1* cassette, fragments of 3′ UTR of *ALS3*, and *ALS3* ORF plus 5′ UTR were used as the templates for fusion PCR. Primers used for PCRs are listed in Table S2.

To heterologously express *C. albicans ALS3* in *S. cerevisiae*, we first subcloned the *ALS3* coding region into plasmid pYES2 at the KpnI and XbaI sites under the control of the *ADH1* promoter. The *ALS3* coding region fragments were amplified from genomic DNA of *C. albicans* SC5314 by PCR and digested with

KpnI and XbaI enzymes. Plasmid pYES2-*ALS3* was then transformed into *S. cerevisiae* strain (BY4741). The *S. cerevisiae* strain transformed with plasmid pYES2 served as a control.

Recombinant expression and purification of human SAA1 in *E. coli*. The methods of recombinant expression and purification of human SAA1 in *Escherichia coli* were performed as described previously (7). Briefly, the expression plasmid pET28-hSAA1 was transformed into *E. coli* strain Rosetta (DE3). To induce the expression of human SAA1 in *E. coli*, bacterial cells were cultured in LB medium containing 50 mg/liter kanamycin and induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG; Sangon Biotech, Shanghai, China). After 3 h of incubation, bacterial cells were harvested and used for rhSAA1 purification using the His·Bind purification kit (Millipore, Uppsala, Sweden).

Cell aggregation assay. *C. albicans* cells were cultured to logarithmic phase in liquid Lee's glucose, Lee's GlcNAc, or YPD medium at 30°C with shaking. The cell cultures (2 ml) were treated with rhSAA1 (at a final concentration of 40 mg/liter) for 1 h at 30°C with shaking (200 rpm). The culture samples were then subjected to cell aggregation examination. The culture samples were gently shaken before being photographed.

RNA-Seq and quantitative real-time PCR. *C. albicans* cells were grown to logarithmic phase in liquid Lee's glucose at 30°C. Cells were then treated with rhSAA1 (at a final concentration of 40 mg/liter) for 1 h and harvested for RNA extraction using the RNA preparation kit (Thermo Scientific, Vilnius, Lithuania).

RNA-Seq analysis was performed by Berry Genomics Co. (Beijing, China). Approximately 10 million (M) reads were obtained by sequencing each library as described previously (22). Briefly, mRNA was purified from total RNA using oligo(dT) magnetic beads and fragmented into small pieces (200 to 700 bp). The cleaved RNA fragments were primed with random hexamers and used to synthesize the first-strand and second-strand cDNAs. Adapters were ligated to the cDNA fragments to generate a library for sequencing (Illumina HiSeq 2500 V4). Illumina software OLB_1.9.4 was used for base calling. The adapter sequences and low-quality reads were removed from the raw data. Clean reads were mapped to the genome of *C. albicans* SC5314 using TopHat v2.1.1 and Cufflinks (version 2.2.1) software (23). Relative gene expression levels were calculated using the fragments per kilobase per million reads (FPKM) method. The GO enrichment analysis was performed with Gene Ontology Consortium (http://www.geneontology.org/) and carried out according to the online CGD GO Term Finder tool (http://www.candidagenome.org/cgi-bin/GO/goTermFinder). The heatmap and GOplot packages for R were used to visualize clustering (24). Differential expression analyses were conducted using the DESeq2 package from Bioconductor. Two biological replicates were performed for each sample for RNA-Seq analysis.

qRT-PCR assays were performed as described previously (22). Briefly, mRNA was first reverse-transcribed into cDNA with RevertAid H Minus reverse transcriptase (Thermo Scientific, Vilnius, Lithuania), and the transcripts were then quantified using a Bio-Rad CFX96 real-time PCR detection system with SYBR green (Toyobo, Osaka, Japan). The relative expression levels of each gene were normalized to that of *C. albicans ACT1*. Primers used for PCR are listed in Table S2.

Killing assays of fungal cells. Antifungal activities of rhSAA1 against the WT and mutant strains of *C. albicans* were determined using a CFU assay as described previously (7). Briefly, fungal cells from a single colony were cultured in liquid YPD medium to logarithmic phase at 30°C with shaking. Cells were collected, washed, and then diluted in Lee's glucose medium to 2×10^5 cells/ml. Fungal cells were then treated with equivoluminal rhSAA1. PBS treatment served as a negative control. Three biological replicates were performed for each test.

rhSAA1 binding assays. Binding assays of rhSAA1 to cells of *S. cerevisiae* were performed according to previous reports (6, 7). Fungal cells (5×10^6 cells/ml) were mixed with 200 mg/liter rhSAA1 at room temperature for 2.5 h. Cells were then washed three times with PBS and incubated with mouse anti-hSAA1 antibody (Abcam, Cambridge, UK) at 4°C overnight. After washing with PBS, Alexa Fluor 488-conjugated goat anti-mouse IgG (Life Technologies, Delhi, India) was then added to the cells for fluorescence microscopy assays. rhSAA1 bound to *S. cerevisiae* cells (1×10^7) was eluted with 6 M urea and detected using an anti-hSAA1 antibody (Abcam, Cambridge, UK).

Data availability. The raw RNA-Seq data have been deposited in the NCBI Gene Expression Omnibus (GEO) portal (accession GSE128598).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.9 MB.

ACKNOWLEDGMENTS

We thank Jun Wu for providing the rhSAA1-expression plasmid.

This work was supported by the National Science and Technology Major Project (http://program.most.gov.cn/; grant number 2018ZX10101004-003-002), National Natural Science Foundation of China awards 31930005 and 31625002 (to G.H.), and funding from the National Institutes of Health (NIH) National Institute of General Medical Sciences (NIGMS) award R35GM124594 (to C.J.N.).

REFERENCES

1. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. 2012. Hidden killers: human fungal infections. Sci Transl Med 4:165rv13. https://doi.org/10.1126/scitranslmed.3004404.
2. Cheng SC, Joosten LAB, Kullberg BJ, Netea MG. 2012. Interplay between *Candida albicans* and the mammalian innate host defense. Infect Immun 80:1304–1313. https://doi.org/10.1128/IAI.06146-11.
3. Uhlar CM, Whitehead AS. 1999. Serum amyloid A, the major vertebrate acute-phase reactant. Eur J Biochem 265:501–523. https://doi.org/10.1046/j.1432-1327.1999.00657.x.
4. Hirakura Y, Carreras I, Sipe JD, Kagan BL. 2002. Channel formation by serum amyloid A: a potential mechanism for amyloid pathogenesis and host defense. Amyloid 9:13–23. https://doi.org/10.3109/13506120209072440.
5. Eckhardt ER, Witta J, Zhong J, Arsenescu R, Arsenescu V, Wang Y, Ghoshal S, de Beer MC, de Beer FC, de Villiers WJ. 2010. Intestinal epithelial serum amyloid A modulates bacterial growth *in vitro* and pro-inflammatory responses in mouse experimental colitis. BMC Gastroenterol 10:133. https://doi.org/10.1186/1471-230X-10-133.
6. Hari-Dass R, Shah C, Meyer DJ, Raynes JG. 2005. Serum amyloid A protein binds to outer membrane protein A of Gram-negative bacteria. J Biol Chem 280:18562–18567. https://doi.org/10.1074/jbc.M500490200.
7. Gong J, Wu J, Ikeh M, Tao L, Zhang Y, Bing J, Nobile CJ, Huang G. 2019. Antifungal activity of mammalian serum amyloid A1 against *Candida albicans*. Antimicrob Agents Chemother 64:e01975-19. https://doi.org/10.1128/AAC.01975-19.
8. Lu J, Yu Y, Zhu I, Cheng Y, Sun PD. 2014. Structural mechanism of serum amyloid A-mediated inflammatory amyloidosis. Proc Natl Acad Sci U S A 111:5189–5194. https://doi.org/10.1073/pnas.1322357111.
9. Noble SM, French S, Kohn LA, Chen V, Johnson AD. 2010. Systematic screens of a *Candida albicans* homozygous deletion library decouple morphogenetic switching and pathogenicity. Nat Genet 42:590–598. https://doi.org/10.1038/ng.605.
10. Hoyer LL. 2001. The ALS gene family of *Candida albicans*. Trends Microbiol 9:176–180. https://doi.org/10.1016/s0966-842x(01)01984-9.
11. Otoo HN, Lee KG, Qiu W, Lipke PN. 2008. *Candida albicans* Als adhesins have conserved amyloid-forming sequences. Eukaryot Cell 7:776–782. https://doi.org/10.1128/EC.00309-07.
12. Grubb SE, Murdoch C, Sudbery PE, Saville SP, Lopez-Ribot JL, Thornhill MH. 2008. *Candida albicans*-endothelial cell interactions: a key step in the pathogenesis of systemic candidiasis. Infect Immun 76:4370–4377. https://doi.org/10.1128/IAI.00332-08.
13. Nobile CJ, Fox EP, Nett JE, Sorrells TR, Mitrovich QM, Hernday AD, Tuch BB, Andes DR, Johnson AD. 2012. A recently evolved transcriptional network controls biofilm development in *Candida albicans*. Cell 148:126–138. https://doi.org/10.1016/j.cell.2011.10.048.
14. Guan G, Xie J, Tao L, Nobile CJ, Sun Y, Cao C, Tong Y, Huang G. 2013. Bcr1 plays a central role in the regulation of opaque cell filamentation in *Candida albicans*. Mol Microbiol 89:732–750. https://doi.org/10.1111/mmi.12310.
15. Filler SG. 2006. *Candida*-host cell receptor-ligand interactions. Curr Opin Microbiol 9:333–339. https://doi.org/10.1016/j.mib.2006.06.005.
16. Zhang LJ, Gallo RL. 2016. Antimicrobial peptides. Curr Biol 26:R14–R19. https://doi.org/10.1016/j.cub.2015.11.017.
17. Phan QT, Myers CL, Fu Y, Sheppard DC, Yeaman MR, Welch WH, Ibrahim AS, Edwards JE, Jr, Filler SG. 2007. Als3 is a *Candida albicans* invasin that binds to cadherins and induces endocytosis by host cells. PLoS Biol 5:e64. https://doi.org/10.1371/journal.pbio.0050064.
18. Zelante T, Iannitti RG, De Luca A, Arroyo J, Blanco N, Servillo G, Sanglard D, Reichard U, Palmer GE, Latge JP, Puccetti P, Romani L. 2012. Sensing of mammalian IL-17A regulates fungal adaptation and virulence. Nat Commun 3:683. https://doi.org/10.1038/ncomms1685.
19. Noble SM, Johnson AD. 2005. Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen *Candida albicans*. Eukaryot Cell 4:298–309. https://doi.org/10.1128/EC.4.2.298-309.2005.
20. Wilson RB, Davis D, Mitchell AP. 1999. Rapid hypothesis testing with *Candida albicans* through gene disruption with short homology regions. J Bacteriol 181:1868–1874. https://doi.org/10.1128/JB.181.6.1868-1874.1999.
21. Reuss O, Vik A, Kolter R, Morschhauser J. 2004. The SAT1 flipper, an optimized tool for gene disruption in *Candida albicans*. Gene 341:119–127. https://doi.org/10.1016/j.gene.2004.06.021.
22. Tao L, Zhang Y, Fan S, Nobile CJ, Guan G, Huang G. 2017. Integration of the tricarboxylic acid (TCA) cycle with cAMP signaling and Sfl2 pathways in the regulation of CO_2 sensing and hyphal development in *Candida albicans*. PLoS Genet 13:e1006949. https://doi.org/10.1371/journal.pgen.1006949.
23. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, Pimentel H, Salzberg SL, Rinn JL, Pachter L. 2012. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc 7:562–578. https://doi.org/10.1038/nprot.2012.016.
24. Walter W, Sanchez-Cabo F, Ricote M. 2015. GOplot: an R package for visually combining expression data with functional analysis. Bioinformatics 31:2912–2914. https://doi.org/10.1093/bioinformatics/btv300.