Infection and Drug Resistance

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ORIGINAL RESEARCH

Roles of Nucleolar Factor *RCL1* in Itraconazole Resistance of Clinical *Candida albicans* Under Different Stress Conditions

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Purpose: RNA terminal phosphate cyclase like 1 (*RCL1*) undergoes overexpression during the immune response of *Candida albicans* following drug treatment. This study aims to investigate the expression levels of *RCL1* in *C. albicans* under various stress conditions.
Methods: Fifteen itraconazole (ITR)-resistant strains of clinical *C. albicans*, and one standard strain were employed for *RCL1* sequencing, and mutations in *RCL1* were analyzed. Subsequently, 14 out of the 15 ITR-resistant clinical strains and 14 clinical strains sensitive to ITR, fluconazole (FCA) as well as voriconazole (VRC) were cultured under diverse conditions. The expression of *RCL1* ITR-resistant and sensitive *C. albicans* was then assessed using real-time quantitative PCR (RT-qPCR) assays.
Results: Compared to the standard strain, three missense mutations (C6A, G10A, and A11T) were identified in the *RCL1* gene of ITR-resistant *C. albicans* through successful forward sequencing. Additionally, using successful reverse sequencing, one synonymous mutation (C1T) and four missense mutations (C1T, A3T, A7G, and T8G) were found in the *RCL1* gene of ITR-resistant *C. albicans*. *RCL1* expression was significantly higher in ITR-resistant *C. albicans* than in sensitive strains under standard conditions (37°C, 0.03% CO_2, pH 4.0). Low temperature (25°C) increased *RCL1* expression in sensitive *C. albicans* while decreasing it in ITR-resistant strains. Elevated CO_2 concentrations (5% CO_2) had a negligible effect on *RCL1* expression in sensitive *C. albicans*, but effectively reduced *RCL1* level in ITR-resistant strains. Furthermore, a medium with a pH of 7 decreased the expression of *RCL1* in both resistant and sensitive *C. albicans*.
Conclusion: This study demonstrated that *RCL1* mutations in ITR-resistant *C. albicans*, and variations in culture conditions significantly influence *RCL1* expression in both ITR-resistant and sensitive *C. albicans*, thereby inducing alterations in the dimorphism of *C. albicans*.
Keywords: *Candida albicans*, itraconazole resistance, *RCL1*, culture conditions

Introduction

Invasive Candidiasis (IC) is a prevalent fungal infection associated with a high mortality rate of 70%. The manifestations of IC range from candidemia to fulminant sepsis, exhibiting varying degrees of severity.[1,2] Several factors, such as prolonged non-standard and high-dose application of immunosuppressants and glucocorticoids, an increase in acquired human immunodeficiency virus (HIV) infections, and the use of various catheters, stents, and other medical instruments, contribute to the annual rise in IC incidence.[3] IC typically involves the invasion of the bloodstream by candida fungi, including species such as *Candida albicans, Candida parapsilosis*, and *Candida tropicalis*. *C. albicans* remains the primary pathogen causing IC.[1,2]

C. albicans, a conditionally pathogenic fungus, can co-exist in healthy individuals as a part of the normal microbiota without causing disease.[4] It commonly resides on mucosal surfaces, like the oral, vaginal, and gastrointestinal mucosa.[5] However, under certain conditions, *C. albicans* can cause a spectrum of diseases, ranging from superficial mucous membranes infections to deep-seated systemic invasions, potentially leading to life-threatening deep fungal infections upon entering the bloodstream.[6] Previous studies have indicated that a variety of factors, such as

Received: 17 July 2023
Accepted: 2 February 2024
Published: 27 February 2024

the transformation of yeast to mycelial morphology, phenotypic transformation, biofilm formation, and the expression of cell surface adhesins, enhance the pathogenicity of *C. albicans*.[7–9] Antifungal drugs, including itraconazole (ITR), fluconazole (FCA), and voriconazole (VRC), are effective against *C. albicans*. However, the abuse and prolonged use of these drugs have led to the emergence of drug-resistant strains, posing a significant challenge to the treatment of *C. albicans* infections.[10] Therefore, it is imperative to elucidate the mechanisms of resistance in *C. albicans* to improve the prognosis of patients.

Ribosomes are arguably the most critical macromolecular machines, tasked with carrying out protein synthesis in cells. Ribosome biogenesis stands out as one of the most crucial and energy-consuming processes within cells.[11] Mature ribosomes consist of chemically modified components, including methylation of the ribose sugars at the C2-position, isomerization of uridines to pseudouridines, and various base modifications in both ribosomal RNA (rRNA) and ribosomal protein (RP).[11] In eukaryotes, ribosomes are composed of a small subunit (40Ss) and a large subunit (60S). The process of ribosome biogenesis is complex and subject to strict regulation to ensure the correct production of ribosomes under various environmental and metabolic changes.[12] Ribosome biogenesis begins with the transcription of rRNA in the nucleolus, and then the transcribed rRNA undergoes complicated folding, modification, processing, and binding to RP. During this process, precursor rRNA (pre-rRNA) is exported from the nucleus to the cytoplasm, eventually maturing into ribosomes with translational activity.[13,14] Over 200 ribosome assembly factors (AFs) and 80 small nucleolar RNAs (snoRNAs) are associated with ribosome biogenesis. Among them, GTPase BMS1 (BMS1) is the sole known GTPase required for the biosynthesis of the 40S ribosome subunit, interacting with RNA terminal phosphate cyclase like 1 (RCL1). RCL1, an RNA 3'-terminal phosphate cyclase, plays a crucial role in the biogenesis of 18S rRNA and pre-rRNA in eukaryotes.[15–17] Identified as a stress gene, *RCL1* responds more rapidly to drugs compared to other ribosomal genes.[18] In our previous studies, we uncovered that *RCL1* is overexpressed *in C. albicans* during the immune response to drug treatment.[17] Differential expression analysis and protein–protein interaction (PPI) network analysis of the CAF-21 expression data file (GSE65396) in *C. albicans* before and after treatment with the macrocyclic compound (RF59) in the GEO database revealed a notable increase in *RCL1* and other ribosome-related genes following drug administration.[17] However, the physiological significance of *RCL1* in Candidal cells, particularly in drug-resistant strains and under different stress conditions, as well as how changes in RCL1 expression may impact the organism and/or the host in an infective situation remain unknown.

The conditions typically employed for the cultivation of C. albicans involve a temperature of 37°C, a CO_2 concentration of 0.03% CO_2 in the air, and a pH level of 4. It is noteworthy that the female vagina is characterized by weak acidity, with a pH range of 3.8–4.4 under normal circumstances. These specified conditions are considered standard and are henceforth referred to as common or normal conditions in this study. The primary objective of this investigation is to assess the impact of RCL1 on drug resistance in C. albicans through an examination of gene expression patterns. Concurrently, C. albicans was subjected to cultivation under diverse conditions to evaluate RCL1 expression and elucidate the role of RCL1 in the face of varying stressors.

Materials and Methods

Experimental Strains

The 15 strains of clinical ITR-resistant *C. albicans* and 1 strain of standard *C. albicans* were utilized for the Sanger sequencing. The quantitative real-time polymerase chain reaction (qRT-PCR) assays were conducted on 14 of the aforementioned 15 strains of clinical ITR-resistant *C. albicans* (the same strains used in sequencing), 14 strains of clinical *C. albicans* sensitive to ITR, FCA, and VRC (sensitive *C. albicans*), and 1 strain of standard *C. albicans* (the same strains used in sequencing). The standard strain (ATCC11006) was procured from the Center for Mycology and Mycosis Research, Peking University Health Science Center. All the clinical strains were isolated, identified, and preserved at the Fungal Laboratory, Department of Dermatology and Venereology, Second Hospital of Shanxi Medical University, China. This study received approval from the Ethics Committee of the Second Hospital of Shanxi Medical University.

DNA Extraction, PCR Amplification, and *RCL1* Sequencing

The strains of *C. albicans* were cultured in Yeast Extract Peptone Dextrose (YPD) Liquid Medium (AOBOX BIOTECHNOLOGY, Beijing, China) at 37°C with a rotational speed of 200 rpm/min. After 24 hours of culture, total DNA from C. albicans was extracted using a Yeast DNA Extraction kit (Shanghai Sangong Biotechnology Co., LTD, Shanghai, China) following the manufacturer's protocols. The concentration (OD260) and purity (OD260/280) of the isolated DNA were determined using a microplate reader (BK-9622, BIOBASE, Shandong, China). To obtain the full-length sequence of the *RCL1* gene, the upstream and downstream primers for *RCL1* (*RCL1*-Forward-5'-TGGCATCACCTTAGTCGCTG-3', *RCL1*-Reverse-5'-GTAAACTCCCAGCCGAACCA-3') were designed and synthesized by Shanghai Sangong Biotechnology Co., LTD. (Shanghai, China). Subsequently, the *RCL1* sequence was amplified on a PCR system (ProFlex, Applied Biosystems, California, USA) using 2×Hieff® Ultra-Rapid HotStart PCR Master Mix (with Dye) (10157ES03, YESEN, Shanghai, China) following the manufacturer's instructions. The PCR products, with a length of 77 bp, were purified by 1% agarose gel electrophoresis, and sent to Shanghai Sangong Biotechnology Co., Ltd. for sequencing. Mutation sites in the RCL1 sequencing were observed using SnapGene 4.1.8 software (Insightful Science, California, USA).

Culture of *C. albicans* Under Diverse Stress Conditions

Culture of *C. albicans* Under Different Temperatures

Sterile inoculation rings were used to inoculate 14 strains of clinical ITR-resistant *C. albicans* and 14 strains of clinical sensitive *C. albicans* onto YPD solid medium (pH = 4, YPDA Medium, PM2011, Coolaber, Beijing, China). The strains were then incubated in either a 25°C or 37°C incubator (SPX-250B-Z, Shanghai Boxun Industrial Co., LTD, Shanghai, China) with 0.03% CO_2 for 48 hours. Subsequently, a single colony was selected and transferred into YPD liquid medium (pH = 4), shaken, and cultured in a 25°C or 37°C atmosphere with 0.03% CO_2 for an additional 24 hours.

Culture of *C. albicans* Under Different CO_2 Concentrations

The sterile inoculation rings were employed to introduce 14 strains of clinical ITR-resistant *C. albicans* and 14 strains of clinical sensitive *C. albicans* onto YPD solid medium (adjusted to pH = 4). Subsequently, the strains were incubated in a 37°C incubator, with 5% or 0.03% CO_2, for a period of 48 hour. Following incubation, a single colony was selected and transferred into YPD liquid medium (pH = 4), agitated, and cultured in a 37°C environment, under 5% or 0.03% CO_2 for an additional 24 hours.

Culture of *C. albicans* Under Different pH Value

Sterile inoculation rings were employed to inoculate 14 strains each of clinical ITR-resistant *C. albicans* and clinical sensitive *C. albicans* onto YPD solid medium at pH = 7 or pH = 4. Subsequently, the strains were incubated in a 37°C incubator with 0.03% CO_2 for 48 hours. Following this, a single colony was selected and transferred to YPD liquid medium (at pH = 7 or pH = 4), where it was shaken and cultured under a 37°C atmosphere with 0.03% CO_2 for an additional 24 hours.

Real-Time Quantitative PCR (RT-qPCR)

The total RNA from *C. albicans* cultured under various conditions was extracted using the Spin Column Yeast Total RNA Purification Kit (B518657, Shanghai Sangong Biotechnology Co., LTD, Shanghai, China) following the manufacturer's protocols. The MightyScript First-Strand cDNA Synthesis Master Mix (B639251, Shanghai Sangong Biotechnology Co., LTD, Shanghai, China) was employed for reverse transcription of the total RNA into complementary DNA (cDNA) in accordance with the instructions of the manufacturer. Subsequently, qRT-PCR analysis was conducted using 2×SYBR Green PCR Master Mix (SR1110, Solarbio, Beijing, China) as outlined previously.[19] The transcription level of *RCL1* was normalized to *ACTB* (*β-actin*), and the relative expression of *RCL1* was calculated using the $2^{-\Delta\Delta Ct}$ method. The primers used in the qRT-PCR assay were as follows: *RCL1*-Forward-5'-GCCAACAGGATGCGAGGTTA-3', *RCL1*-Reverse-5'- TCAGCGACTA AGGTGATGCC-3'; *ACTB*-Forward-5'-TGTTGACCGAAGCTCCAATGA-3', *ACTB*-Reverse-5'-ACCGGTGGTTCTACC AGAAG-3'.

Statistical Analysis

All data were analyzed using SPSS software (version 26.0, SPSS, Inc., Chicago, IL, USA) and were presented as mean ± standard deviation (SD). The differences between the two groups were assessed using the Student's *t*-test. $*P < 0.05$ indicated a statistical difference.

Results

RCL1 Sequencing Analysis

The *RCL1* gene of 16 strains of *C. albicans* was subjected to bidirectional sequencing in this study. The forward sequencing revealed successful sequencing of six *C. albicans* strains, including the standard strain ATCC11006 and the ITR-resistant strains SY13, SY17, SY18, SY32, and SY35. *RCL1* gene sequences of the five ITR-resistant strains, which were successfully sequenced in the forward direction, were compared to those of the standard strain ATCC11006. Table 1 illustrates that a total of three missense mutations were identified in ITR-resistant *C. albicans*, namely C6A, G10A, and A11T.

Results from reverse sequencing demonstrated the successful sequencing of all 16 strains of *C. albicans*. Comparative analysis with the *RCL1* gene of the standard strain ATCC11006 revealed one synonymous mutation (C1T) and four missense mutations (C1T, A3T, A7G, and T8G) in the 15 ITR-resistant strains that were successfully sequenced in the reverse direction (Table 2).

Table 1 Base Mutation Sites and Amino Acid Mutation for *RCL1* in *Candida albicans* Strains Through Forward Sequencing

No. of Strains	ITR Sensitivity	Base Mutation	Amino Acid Mutation
SY13	RT	Base addition at 6^{th} and 7^{th}	Frameshift mutation
SY17	RT	Base deletion at 1^{st} and base addition at 26^{th}	Frameshift mutation
SY18	RT	Base addition at 1^{st}, 2^{nd} and 28^{th}	Frameshift mutation
SY32	RT	Base deletion at 1^{st} and base addition at 26^{th}	Frameshift mutation
SY35	RT	C6A, G10A, A11T	S6R [a], S10R [a], D11I [a]

Note: [a]Missense mutation.
Abbreviations: RT, resistant to ITR; S, serine; R, arginine; D, aspartic acid; I, isoleucine.

Table 2 Base Mutation Sites and Amino Acid Mutation for *RCL1* in *Candida albicans* Strains Through Reverse Sequencing

No. of Strains	ITR Sensitivity	Base Mutation	Amino Acid Mutation
SY13	RT	Base addition at 1st and base deletion at 9th	Frameshift mutation
SY17	RT	Base addition at 6th	Frameshift mutation
SY18	RT	Base deletion at 1st	Frameshift mutation
SY20	RT	Base addition at 1st	Frameshift mutation
SY25	RT	Base addition at 1st	Frameshift mutation
SY29	RT	C1T, A3T	R1W[a], R3W[a]
SY31	RT	Base deletion at 1st, and 16th	Frameshift mutation
SY32	RT	Base deletion at 1st, 9th	Frameshift mutation
SY35	RT	Base addition at 1st	Frameshift mutation
SY37	RT	Base addition at 1st and base deletion at 29th	Frameshift mutation
SY39	RT	C1T	R1R[b]
SY40	RT	Base deletion at 16th	Frameshift mutation
SY41	RT	Base deletion at 1st	Frameshift mutation
SY43	RT	Base deletion at 5th	Frameshift mutation
SY47	RT	A7G, T8G	Y7W, Y8W

Notes: [a]Missense mutation sites; [b]Synonymous mutation.
Abbreviations: RT, resistant to ITR; R, arginine; W, tryptophan; Y, tyrosine.

Expression of *RCL1* in the Sensitive and ITR-Resistant *C. albicans*

Subsequently, we determined the expression levels of *RCL1* in both susceptible and ITR-resistant strains of *C. albicans*. The data revealed a significant upregulation of *RCL1* expression in ITR-resistant *C. albicans* compared to the susceptible strain under normal conditions (37°C, 0.03% CO_2, pH 4.0) ($P < 0.05$, Figure 1A). At a lower temperature (25°C, 0.03% CO_2, pH = 4.0), the expression levels of *RCL1* in both sensitive and ITR-resistant *C. albicans* were comparable, with no statistically significant difference ($P > 0.05$, Figure 1B). Although the expression of *RCL1* in ITR-resistant *C. albicans* was slightly elevated with increased CO_2 concentration (37°C, 5% CO_2, pH 4.0), the difference did not reach statistical significance ($P > 0.05$, Figure 1C). Intriguingly, the expression of RCL1 in ITR-resistant *C. albicans* was notably higher than in sensitive strains when the pH of the culture conditions was increased to 7.0 (37°C, 0.03% CO_2, pH 7.0) ($P < 0.05$, Figure 1D). These findings suggest that *RCL1* expression varies between sensitive and resistant *C. albicans* strains under different culture conditions.

Expression of *RCL1* in *C. albicans* Under Diverse Temperature Conditions

The expression levels of *RCL1* in *C. albicans* under various temperature conditions were determined using qRT-PCR. In the sensitive strains of *C. albicans, RCL1* expression at 25°C was significantly higher than that at 37°C ($P < 0.05$, Figure 2A). In contrast, the level of RCL1 was remarkably lower at 25°C compared to 37°C in the ITR-resistant strains of *C. albicans* ($P < 0.05$, Figure 2B). These data indicate that temperature could influence the expression of *RCL1* in both the sensitive and resistant strains of *C. albicans*.

Expression of *RCL1* in *C. albicans* Under Diverse CO_2 Conditions

The *RCL1* expression in *C. albicans* was investigated under different CO_2 conditions. Figure 3A illustrates that an increased CO_2 concentration (5% CO_2) had minimal impact on *RCL1* expression in the sensitive strains of *C. albicans* ($P > 0.05$). However, in ITR-resistant *C. albicans*, the *RCL1* level significantly decreased under 5% CO_2 conditions compared to 0.03% CO_2 conditions ($P < 0.05$, Figure 3B). These results demonstrate that CO_2 concentration influences the expression of *RCL1* in the resistant *C. albicans* but not in the sensitive *C. albicans*.

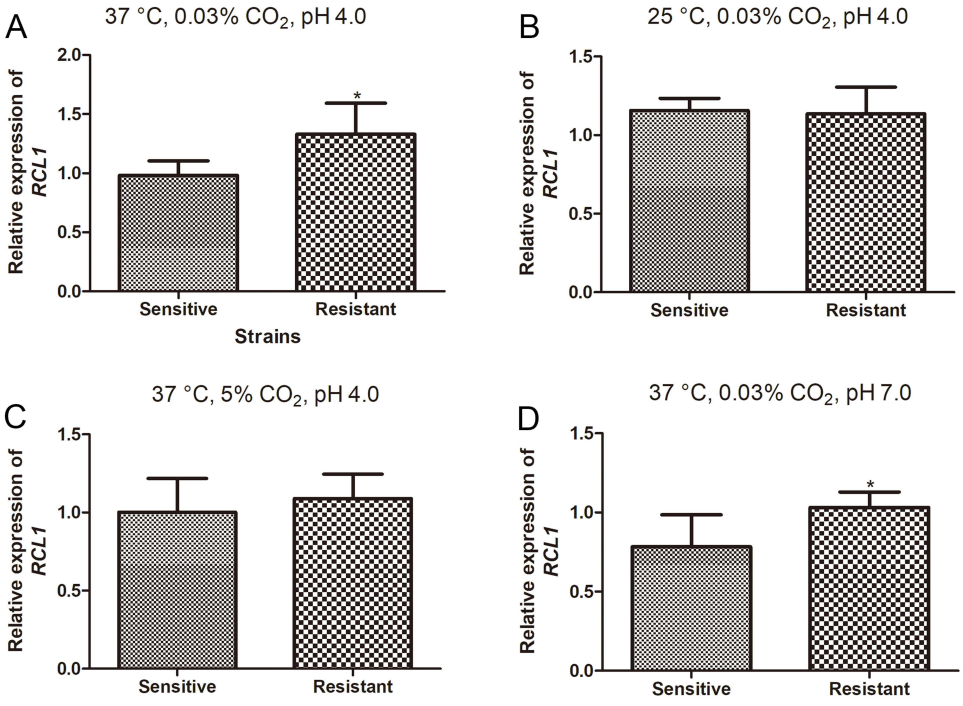

Figure 1 Expression of *RCL1* in the sensitive and resistant *C. albicans*. (**A**) *RCL1* expression in sensitive and ITR-resistant *C. albicans* in normal condition (37°C, 5% CO_2, pH = 4.0). N = 14. (**B**) *RCL1* expression in sensitive and ITR-resistant *C. albicans* at low temperature (25°C, 0.03% CO_2, pH = 4.0). N = 14. (**C**) The level of RCL1 in sensitive and ITR-resistant *C. albicans* at high CO_2 concentration (37°C, 5% CO_2, pH = 4.0). N = 14. (**D**) The expression of *RCL1* in sensitive and ITR-sensitive *C. albicans* in neutral conditions (37°C, 0.03% CO_2, pH = 7.0). N = 14. *P < 005, compared with the sensitive strains.

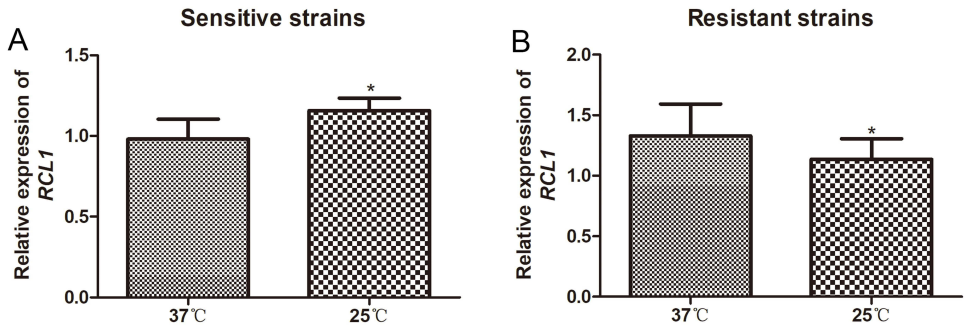

Figure 2 Expression of *RCL1* in C. albicans under diverse temperature conditions. (**A**) *RCL1* expression in sensitive *C. albicans* at 37°C or 25°C. N = 14. (**B**) *RCL1* expression in ITR-resistant *C. albicans* at 37°C or 25°C. N = 14. *P < 005, compared with the strains cultured at 37°C.

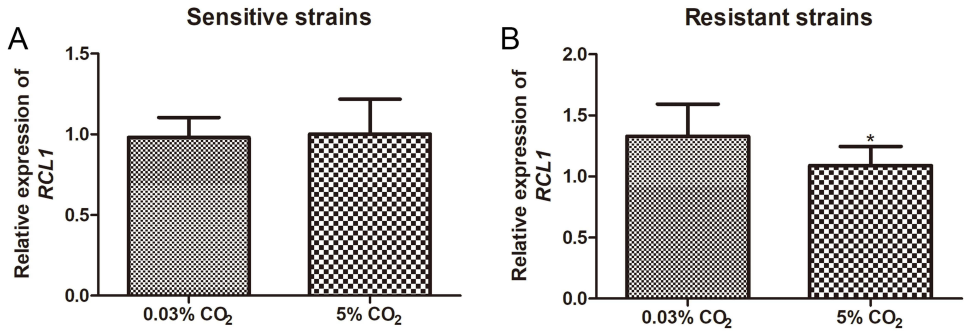

Figure 3 Expression of *RCL1* in *C. albicans* under diverse CO_2 condition. (**A**) The RCL1 level in sensitive *C. albicans* under 0.03% or 5% CO_2 concentration. N = 14. (**B**) The *RCL1* expression in ITR-resistant *C. albicans* under 0.03% or 5% CO_2 concentration. N = 14. *P < 005, compared with the strains cultured with 0.03% CO_2.

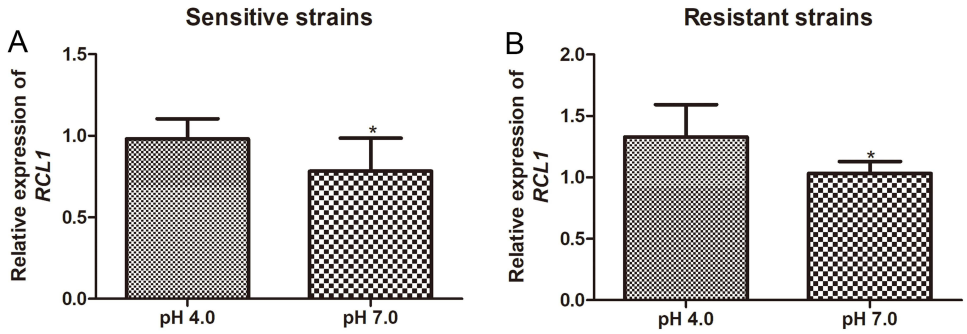

Figure 4 Expression of *RCL1* in *C. albicans* under different pH condition. (**A**) The *RCL1* expression in sensitive *C. albicans* under pH = 4 or pH = 7 condition. N = 14. (**B**) The *RCL1* expression in ITR-resistant *C. albicans* under pH = 4 or pH = 7 condition. N = 14. *P < 005, compared with the strains cultured at pH = 4.

Expression of *RCL1* in *C. albicans* Under Diverse pH Conditions

Further analyses revealed that an elevated pH value (pH = 7.0) significantly diminished the expression levels of *RCL1* in both the sensitive and ITR-resistant strains of *C. albicans* ($P < 0.05$, Figure 4A and B). These findings suggest that distinct pH values modulate *RCL1* expression in both the sensitive and resistant strains of *C. albicans*.

Discussion

C. albicans is a common human pathogen, and IC caused by this fungus poses a threat to the lives and health of patients.[20] RCL1 is a phosphate cyclase closely associated with the processing of rRNA.[21] Growing evidence indicates that RCL1 can bind to BMS1, a typical GTPase involved in the synthesis of 18S rRNA.[22] It has been reported that the RCL1–BMS1 complex plays a crucial role in processing yeast pre-rRNAs.[23] Moreover, our previous study demonstrated

a significant increase in *RCL1* expression in *C. albicans* upon treatment with RF59, a macrocyclic compound.[17] These findings suggest that *RCL1* contributes to the improved the physiological status of *C. albicans*. However, the expression *1* changes of *RCL* in *C. albicans* have not been thoroughly investigated. In this study, we conducted *RCL1* sequencing on 16 *C. albicans* isolates, revealing mutation sites in the *RCL1* gene in ITR-resistant *C. albicans* compared to the standard strain. Specifically, among the five successfully sequenced ITR-resistant strains, three missense mutations were identified in the RCL1 sequence: C6A, G10A, and A11T. Reverse sequencing of 15 successfully sequenced ITR-resistant strains unveiled one synonymous mutation (C1T) and four missense mutations (C1T, A3T, A7G, and T8G) in the *RCL1* sequencing. These results suggest that reverse sequencing detected more mutations than forward sequencing, potentially due to base mutations.[24] The full length of the *RCL1* gene in our study was 77 bp, which may not be sufficient, increasing the likelihood of base mutations.[25] Additionally, mutations were only identified in ITR-resistant *C. albicans*, indicating a potential association between RCL1 mutations and ITR resistance. However, further studies are needed to validate the correlation between *RCL1* mutations and ITR resistance.

Stress responses involve various mechanisms that enable microorganisms to survive in adverse and fluctuating environments.[9] Microorganisms typically possess intricate "circuits" capable of responding to diverse stresses, exemplified by the response of Candida to antifungal drugs.[26] There is a growing body of evidence indicating that C. albicans exhibits different cell morphologies under varying conditions, including "white, opaque, gray", "yeast state", and "mycelium state". These cell morphologies could be converted to each other, which endows *C. albicans* with diverse pathogenic abilities.[27] Taken together, we speculated that stress may exert an essential role in the drug resistance of *C. albicans*.

Additionally, stress often leads to the reorganization of the nuclear structure. The nucleolus serves as a stress sensor, coordinating the stress response.[28] Recently, accumulating evidence has demonstrated that damage to the integrity of the nucleolar system and disruption of ribosome generation can activate the nucleolar stress signaling pathway, ultimately compromising its role in cell growth, apoptosis, and DNA regulation.[29] The viral stress gene RCL1 encoding a nucleolar protein is essential for 18S rRNA in ribosome biosynthesis.[15,17] Moreover, Enjalbert et al verified that RCL1 is the main regulatory factor in *C. albicans* under various stress conditions, including oxidation and heavy metal stress.[18] As *C. albicans* is well known for its characteristic dimorphism, existing as both a yeast and mycelium form, different environmental stimuli, such as a neutral pH value, 25°C and 5% CO_2 can induce changes in its dimorphism of *C. albicans*.[30,31] The transition from yeast to mycelia is a crucial virulence factor in *C. albicans*.[32] However, the impact of different culture environments (temperature, CO_2 concentration, and pH value) on the expression of RCL1 in *C. albicans* remains unclear.

In this study, we investigated the expression of RCL1 in *C. albicans* under normal conditions (37°C, 0.03% CO_2, pH 4.0). We observed higher *RCL1* expression in ITR-resistant *C. albicans* compared to sensitive *C. albicans*. The decrease in temperature to 25°C and the increase in CO_2 concentration to 5% CO_2 both led to similar *RCL1* expression levels in both sensitive and resistant strains of *C. albicans*. Notably, an up-regulated pH value (pH = 7.0) resulted in increased *RCL1* expression specifically in the ITR-resistant *C. albicans* compared to the sensitive *C. albicans*. These findings suggest that *RCL1* expression is inherently higher in resistant *C. albicans* under normal conditions. Interestingly, alterations in temperature and CO_2 concentration effectively eliminated the differences in RCL1 expression levels between sensitive and resistant *C. albicans*.

Subsequent experiments revealed that, when compared to 37°C, a temperature of 25°C led to a significant increase in the RCL1 level in sensitive *C. albicans*, while concurrently decreasing it in ITR-resistant *C. albicans*. Conversely, exposure to 5% CO_2 showed no significant effect on RCL1 content in sensitive *C. albicans*, in contrast to the notable reduction observed in ITR-resistant strains, when compared to 0.03% CO_2. Furthermore, an increase in pH was found to up-regulate *RCL1* expression in both sensitive and resistant *C. albicans*.

This study had certain limitations. A significant constraint is the absence of cross-condition testing, which is essential for establishing the effects of stressors (temperature, CO_2, and pH) on the expression of RCL1. The findings indicate a notable increase in RCL1 expression in ITR-resistant *C. albicans* was notably higher compared to sensitive strains, suggesting a potential involvement of RCL1 in ITR resistance. However, this conclusion warrants further exploration in future studies. Furthermore, additional experiments will be conducted to investigate whether various conditions (temperature, CO_2 concentration, and pH) regulate the morphological transformation and pathogenicity of *C. albicans* by influencing *RCL1* expression.

Conclusion

In summary, this study unveils that ITR-resistant *C. albicans* harbors several mutation sites in the *RCL1* gene. Forward sequencing identified three missense mutations (C6A, G10A, and A11T) in the five strains of ITR-resistant *C. albicans*, while reverse sequencing revealed one synonym mutation (C1T) and four missense mutations (C1T, A3T, A7G, and T8G) among the 15 strains of ITR-resistant *C. albicans*. Different stress conditions (25°C, 5% CO_2, pH = 7.0) exert distinct regulatory effects on *RCL1* expression in both sensitive and resistant *C. albicans* strains. These findings suggest that *C. albicans* may activate its stress response through the RCL1 protein, providing a novel perspective for understanding the molecular targets of potential antifungal drugs for treating patients with invasive candidiasis.

Data Sharing Statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Funding

This study was supported by the General Project of National Natural Science Foundation of China (Project number: 82072262), Scientific and Technological Activities funding program for Overseas Students of Shanxi Province (Project number: 20210030), Research Project Supported by Shanxi Scholarship Council of China (Project number: 2020–190), Natural Science Foundation of Shanxi Province (Project number: 202103021224416), and Shanxi Province colleges and universities excellent achievement cultivation project (Project number: 2019KJ025).

Disclosure

The authors declare that they have no conflicts of interest in this work.

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