Screening of potential genes contributing to the macrocycle drug resistance of *C. albicans* via microarray analysis

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Abstract. The aim of the present study was to investigate the potential genes involved in drug resistance of *Candida albicans* (*C. albicans*) by performing microarray analysis. The gene expression profile of GSE65396 was downloaded from the Gene Expression Omnibus, including a control, 15-min and 45-min macrocyclic compound RF59-treated group with three repeats for each. Following preprocessing using RAM, the differentially expressed genes (DEGs) were screened using the Limma package. Subsequently, the Kyoto Encyclopedia of Genes and Genomes pathways of these genes were analyzed using the Database for Annotation, Visualization and Integrated Discovery. Based on interactions estimated by the Search Tool for Retrieval of Interacting Gene, the protein-protein interaction (PPI) network was visualized using Cytoscape. Subnetwork analysis was performed using ReactomeFI. A total of 154 upregulated and 27 downregulated DEGs were identified in the 15-min treated group, compared with the control, and 235 upregulated and 233 downregulated DEGs were identified in the 45-min treated group, compared with the control. The upregulated DEGs were significantly enriched in the ribosome pathway. Based on the PPI network, PRP5, RCL1, NOP13, NOP4 and MRT4 were the top five nodes in the 15-min treated comparison. GIS2, URA3, NOP58, ELP3 and PLP7 were the top five nodes in the 45-min treated comparison, and its subnetwork was significantly enriched in the ribosome pathway. The macrocyclic compound RF59 had a notable effect on the ribosome and its associated pathways of *C. albicans*. RCL1, NOP4, MRT4, GIS2 and NOP58 may be important in RF59-resistance.

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Introduction

Candida albicans (*C. albicans*) is a common commensal fungus which is normally accumulated in the oral cavity, respiratory tract and gastrointestinal systems in low numbers in healthy individuals (1). *C. albicans* is one of the most frequent opportunistic fungal pathogens of candidiasis. According to data provided by the US National Health Care Safety Network, *C. albicans* is the fourth most common microorganism infection in US hospitals (2). It has been estimated that ~250,000-400,000 individuals succumb to *C. albicans*-associated mortality annually worldwide (3). Although there are several antifungal drugs available, including azoles, polyenes, allylamines and echinocandins (4), the efficiency of anti-infection therapy remains low and drug resistance is a predominant problem in clinical therapy (5). Therefore, it is essential to elucidatethe mechanism of *C. albicans* infection in order to provide novel insights into the clinical treatment of candidiasis.

Following the identification of *C. albicans*, studies have investigated the processes involved in its infection and methods against infection. Generally, candidiasis is not detected in healthy individuals but is frequently detected in individuals who are immunocompromised to a certain extent, including babies, children, the elderly and patients suffering from immune system disease (6). Raska *et al* reported that the virulence factors secreted by *C. albicans* are one of the main factors enabling them to damage tissues and evade the immune system of the host (7). It has also been demonstrated that the transition of a yeast-like phenotype to a hyphal phenotype is an essential procedure for infection (8). pH, temperature and the ratio of oxygen/carbon dioxide in the microenvironment are also crucial in the process of *C. albicans* infection (9). Exposure to antifungal drugs is the main therapeutic approach for the candidiasis, however, due to thee overuse of anti-fungal drugs and the adaptive alteration of fungal genomes,

the efficiency of clinical treatment remains low and the development of novel methods has been slow (10). Although, several studies have focused on the relevant factors, including cytokines, chemokines and effector cells, involved to obtain further insight into this process, information is lacking due to the restricted analysis (9). Therefore, it is important to gain a wider understanding of *C. albicans*.

To further analyze the alterations to the *C. albicans* transcriptome following treatment with macrocyclic compound FR59, Sanglard *et al* (10) generated a microarray of *C. albicans* (GSE65396) treated with 3 μM FR59 at the two time points of 15 and 45 min (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE65396). In this microarray analysis, Sanglard *et al* showed that FR59 may induce a reduction in the expression of genes involved in cell wall biosynthesis, and that ATP binding cassette transporters may be involved in this process. This indicates that FR59 may be a promising drug for the treatment of candidiasis. However, the detailed reasons for this alteration to the cell wall remain to be elucidated. In order to obtain amore detailed understanding of *C. albicans* treated with FR59, the present study performed further analysis of the GSE65396 microarray. By performing this analysis, potential target genes or biofunctions may be identified, which may provide novel insights into the treatment of candidiasis.

Materials and methods

Gene expression profile. The gene expression profile of GSE65396 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE65396) for the macrocyclic compound RF59-treated *C. albicans* was downloaded from the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/), which was sequenced on the Agilent-037331 Copy of *C. albicans* assembly 21_017942 platform. This time profile contained three groups: 0 min control group, 15 min RF59-treated group and 45 min-RF59 treated group, with three repetitions in each.

Data processing. The Affy package (11) in R (Version 3.3.0, http://www.bioconductor.org/packages/release/bioc/html/affy.html) was used to read the raw data, the and RMA (12,13) method was used for data processing, including background correction, normalization and expression calculation. Annotation of the gene expression profile was performed to transform the probe IDs into gene symbols. In cases of different probes mapping to one gene, the mean value was considered to be the final expressed level of this gene.

Identification of differentially expressed genes (DEGs). The Bayes test in the Limma (14) package was utilized to screen the DEGs of the 15 min group, vs. control; and the 45 min group, vs. control, P<0.05 and |log$_2$ fold change >0.585 were considered to be significant.

Pathway enrichment analysis for DEGs. The Database for Annotation, Visualization and Integrated Discovery (DAVID) is a free database which uses an agglomeration algorithm to organize functionally related genes and terms into a manageable number of biological modules (15). Kyoto Encyclopedia

of Genes and Genomes (KEGG) is a another free database for the systematic analysis of gene functions by linking current knowledge to cellular processes (16). In the present study, the DAVID online tool (Version 6.8, https://david-d.ncifcrf.gov/) was used to perform KEGG pathway enrichment analysis of DEGs. P<0.05 was set as the significance cut-off.

Protein-protein interaction (PPI) network. The Search Tool for Retrieval of Interacting Genes (STRING) is a precompute global database for the analysis and exploration of protein interaction (17). To further reveal the biofunctions of the identified DEGs, STRING (version 10.0, http://www.string-db.org/) was used to examine the interactions between differentially expressed proteins. The threshold was set as a required confidence >0.4.

Based on the results of the STRING analysis, Cytoscape (Version 3.2.0, http://www.cytoscape.org/) (18) was utilized to visualize the PPI network of differentially expressed proteins and the CytoNCA plug-in of Cytoscape (19) was used to perform topological analysis, including degree centrality (DC), betweenness centrality (BC) and closeness centrality (CC). Following calculation of the score of each node, the hub proteins in the PPI network were screened out (20).

Functional subnetwork analysis of DEGs. The ReactomeFI plug-in of Cytoscape is an application with a loaded gene expression profile and can be utilized to calculate the weights of edges in a PPI network. In the present study, ReactomeFI was used to compute the correlations as weights for the edges of the PPI network and was combined with the Markov Cluster Algorithm method to map significant functional subnetworks in the PPI network (21). Subsequently the DAVID online tool was used to perform KEGG pathway enrichment analyses for the selected subnetwork. P<0.05 was considered significant.

Results

Screening of DEGs. Following preprocessing and analysis, a total of 181 DEGs were identified in the 15-min treatment group, compared with the control group, which included 154 upregulated and 27 downregulated genes (Fig. 1A). A total of 468 DEGs were screened out in the 45-min treatment group, compared with the control group, which included 235 upregulated and 233 downregulated genes (Fig. 1B).

Functional enrichment analysis of DEGs. To further investigate the biofunctions of the DEGs, KEGG pathway enrichment analysis was performed. The results showed that the upregulated DEGs in the 15-min treatment group were significantly enriched in functions, which included ribosome biogenesis in eukaryotes (P=8.28E-16), oxocarboxylix acid metabolism (P=0.003), and RNA polymerase (P=0.009). The downregulated DEGs were significantly enriched in cyanoamino acid metabolism (P=0.020), as show in Fig. 2. The upregulated DEGs in the 45-min treatment group were significantly enriched in functions, which included ribosome (P=1.03E-10), pyrimidine metabolism (P=2.14E-04) and ribosome biogenesis in eukaryotes (P=4.39E-04). The downregulated DEGs were significantly enriched in functions,
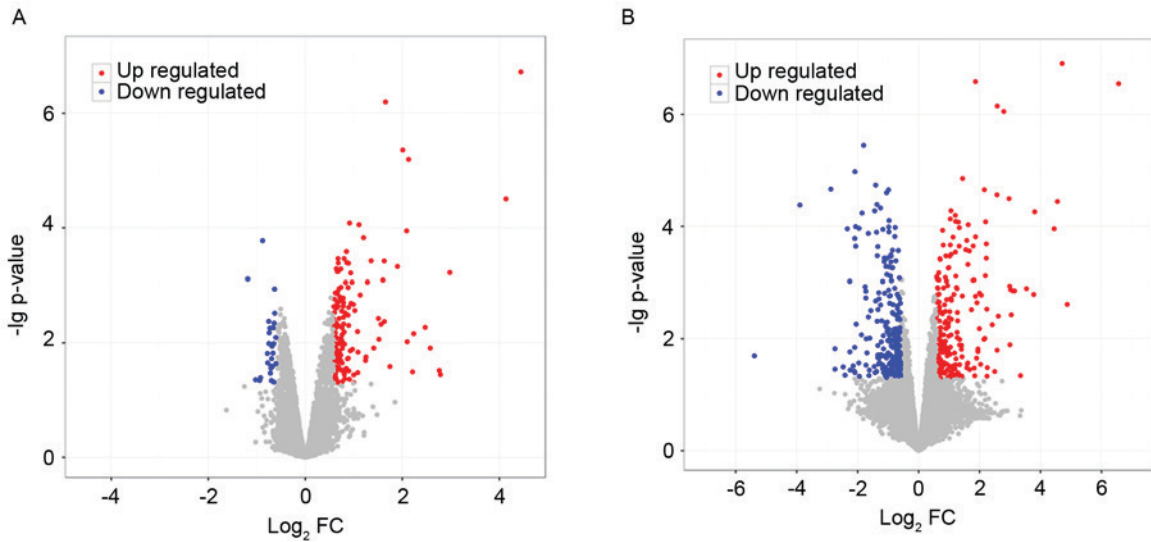
Figure 1. Volcano plots for differentially expressed genes. (A) 15-min RF59 treatment group, vs. control group; (B) 45-min RF59 treatment group, vs. control group. Red represents the upregulated genes and blue represents the downregulated genes. FC, fold change.
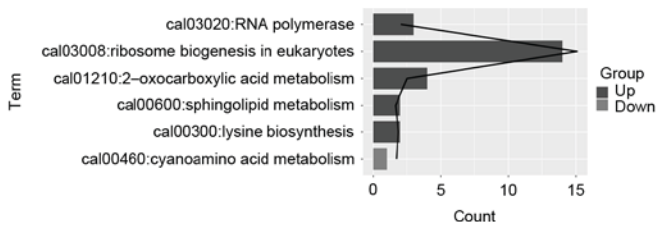

Figure 2. Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis of differentially expressed genes in the 15-min RF59 treatment group, vs. control group. The dark grey represents upregulated genes and the light grey represents downregulated genes. The connected line represents the lg P-value.
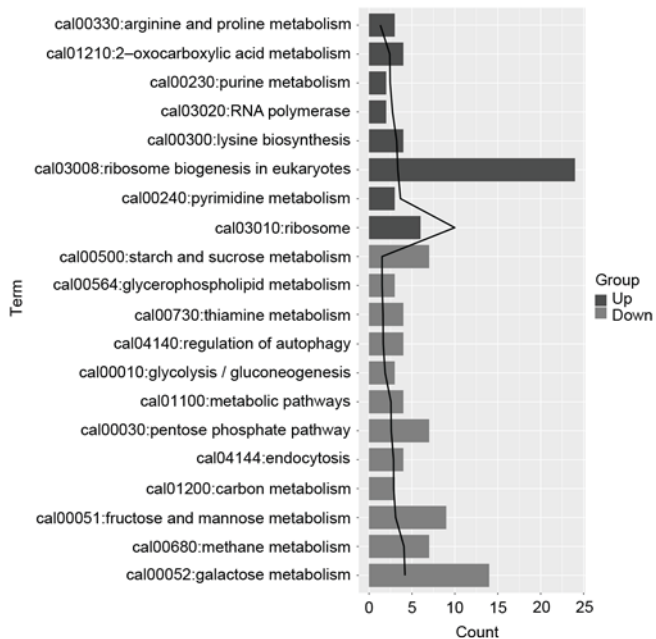

Figure 3. Kyoto Encyclopedia of Genes and Genomes pathway enrichment analytical results of differentially expressed genes in the 45-min RF59 treatment group, vs. control group. The dark grey represents upregulated gene and the light grey represents downregulated genes. The connected line represents the lg P-value.
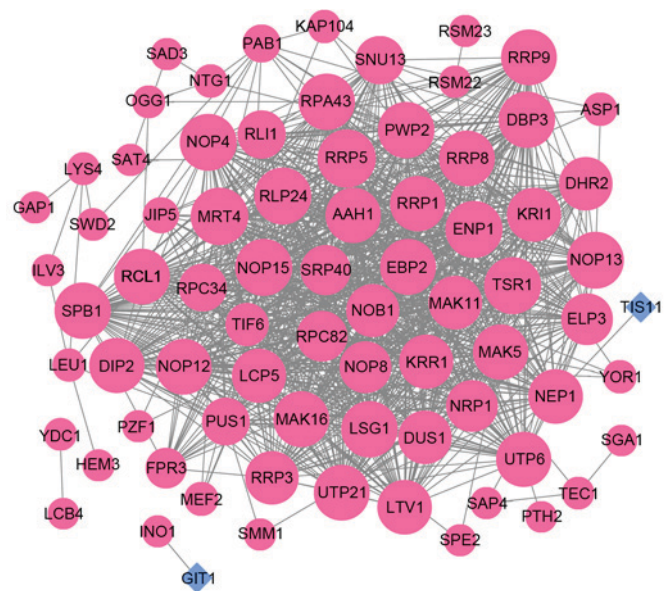

Figure 4. Protein-protein interaction network of the 15-min RF59 treatment group, vs. control group. Pink circles represent upregulated genes and blue diamonds represent downregulated genes. Lines represent edges. The node size is positively correlated with its degree.

which included galactose metabolism (P=6.49E-05), methane metabolism (P=8.45E-05), and fructose and mannose metabolism (P=7.88E-04), as shown in Fig. 3.

PPI network analysis. Due to the deficiency of *C. albicans* data in STRING, the profile of *Saccharomyces cerevisiae* (*S. cerevisiae*) was used. According to the biofunctions of the DEGs, the PPI network of the 15-min treatment group was constructed, which contained 76 nodes and 975 edges (Fig. 4). Based on the scores from the topological analysis, PRP5 (DC=48, BC=273.754, CC=0.185), RCL1 (DC=44, BC=456.142, CC=0.184), NOP13 (DC=46, BC=116.109, CC=0.184), MRT4 (DC=47, BC=87.329, CC=0.184) and NOP4

Table I. Top 15 nodes identified in the topological analysis of the 15-min treatment protein-protein interaction network.

Node	Value
Degree centrality	
RRP5	48
MRT4	47
NOP13	46
ENP1	46
RLP24	46
NOP4	46
TSR1	46
NOP15	46
PWP2	45
RPA43	45
SPB1	45
DBP3	45
LSG1	45
RCL1	44
RRP8	44
Betweenness centrality	
LEU1	499.743
RCL1	456.152
AAH1	315.751
RRP8	311.275
RRP5	273.754
PUS1	260.075
PAB1	186.937
PWP2	182.521
LYS4	149.977
NEP1	147.078
UTP6	146.198
TEC1	141.955
RSM22	140.000
ELP3	136.432
NOP13	116.109
Closeness centrality	
RRP5	0.185185
RCL1	0.184275
NOP13	0.183824
MRT4	0.183824
NOP4	0.183824
TSR1	0.183824
PWP2	0.183374
ENP1	0.183374
RLP24	0.183374
SPB1	0.183374
DBP3	0.183374
NOP15	0.183374
RRP8	0.182927
RPA43	0.182927
LSG1	0.182927

(DC=46, BC=40.931, CC=0.184) were the top five nodes in the PPI network (Table I). The PPI network of the 45-min treatment group was also constructed, containing 177 nodes and 1,051 edges (Fig. 5). According to the scores from the topological analysis, GIS2 (DC=44, BC=4461.883, CC=0.202), URA3 (DC=38, BC=4507.660, CC=0.203), NOP58 (DC=42, BC=806.258, CC=0.200), ELP3 (DC=36, BC=1725.969, CC=0.198) and PLP7 (DC=49, BC=1129.925, CC=0.197) were the top five nodes in the PPI network (Table II). NOP4 and ELP3 were two shared genes within the top 20 of the two comparisons.

Functional subnetwork analysis. Following analysis using the ReactomeFI plugin, a significantly functional subnetwork of the 45-min treatment group was identified with an average correlation of 0.969. In this subnetwork, seven nodes and 16 edges were included (Fig. 6). All of these nodes were upregulated and significantly enriched in the ribosome pathway (P=2.08E-11).

Discussion

According to the analytical criteria, a total of 181 DEGs were identified in the 15-min treatment group, compared with the control group, which included 154 upregulated and 27 downregulated genes. A total of 468 DEGs were identified in the 45-min treatment group, compared with the control group, which included 235 upregulated and 233 downregulated genes. Between these two comparisons, the upregulated DEGs were significantly enriched in ribosome related pathways. Based on the PPI network analysis, PRP5, RCL1, NOP13, MRT4 and NOP4 were the top five nodes in the PPI network of the 15-min treatment group. GIS2, URA3, NOP58, ELP3 and PLP7 were the top five nodes in the PPI network of the 45-min treatment group. NOP58 showed significant involvement in the subnetwork, which was significantly enriched in the ribosome pathway.

Based on the PPI network analysis, RCL1 and MRT4 were two hub nodes in the 15-min treatment group. RCL1 is an RNA 3'-terminal phosphate cyclase, which is similar to *S. cerevisiae* RCL1 involved in rRNA processing (22). It has been demonstrated that RCL1 can interact with an important GTPase, Bms1, having an essential role in the process of 18S rRNA biogenesis (23). In addition, Delprato *et al* reported that the RCL1-Bms1complex is crucial in the pre-ribosomal RNA processing of yeast (24). In the present study, a significant upregulation was identified in *C. albicans* following treatment with RF59 for 15 min. Enjalbert *et al* demonstrated that RCL1 is a core stress genes under oxidative, osmotic and heavy metal stress in *C. albicans* (25). These results indicate that the upregulation of RCL1 may be key in rRNA biosynthetic processing. With this alteration, *C. albicans* may be able to adjust to a physiological status enabling improved response to stress from RF59. MRT4 was also identified as an upregulated node involved in the processing of rRNA in the PPI network of the 15-min treatment group. MRT4 is a non-essential nucleolar protein, which is involved in the biogenesis of the 60S r-subunit (26). Being homologous to the N-terminal domain of P0, Rodríguez-Mateos *et al* reported that MRT4 may have the same rRNA binding site as P0 in the process of ribosome synthesis (27). Rodriguez-Mateos *et al* also showed that MRT4 is a notable nucleolar component of
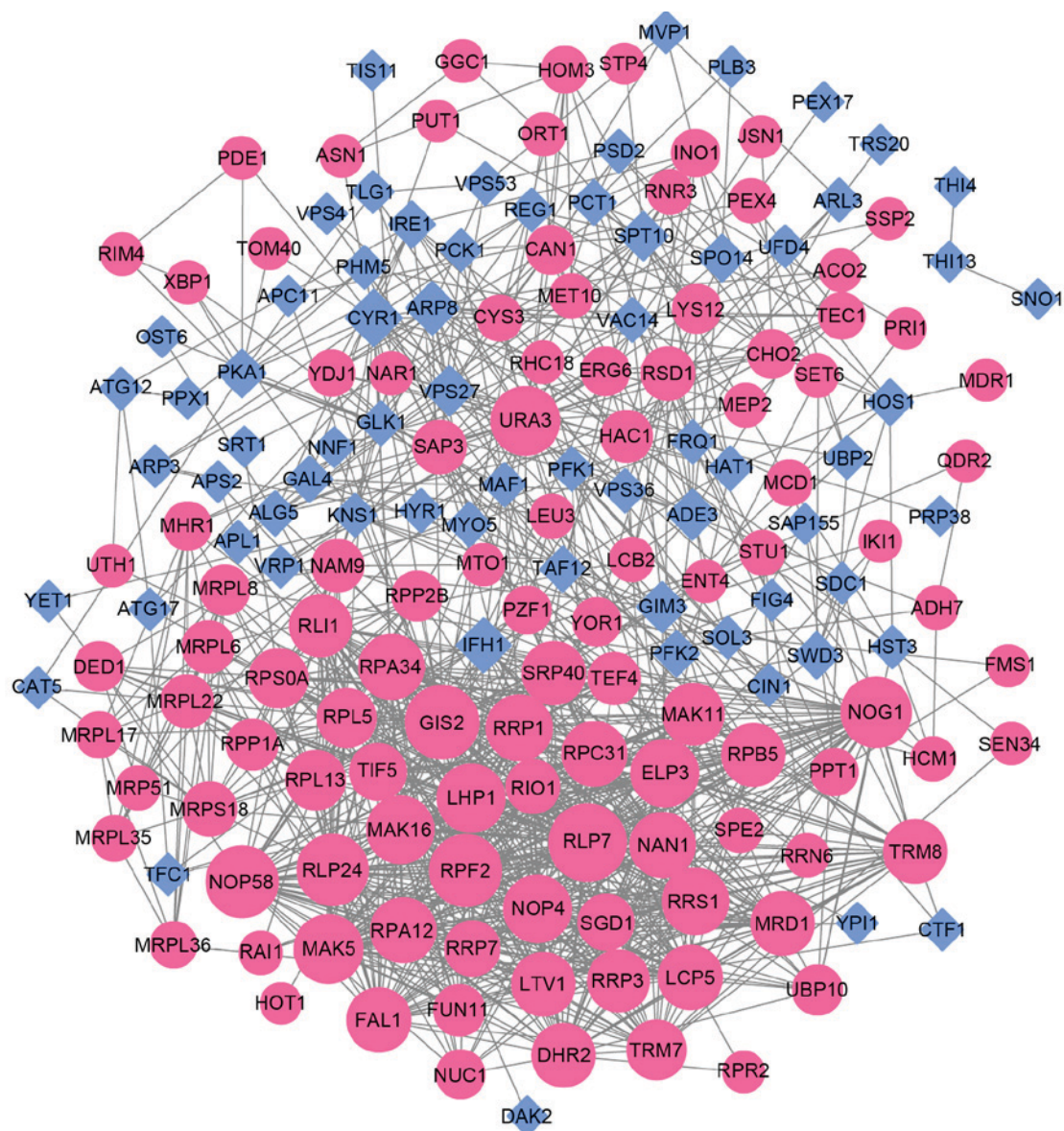
Figure 5. Protein-protein interaction network of the 45-min RF59 treatment group, vs. control group. Pink circles represent upregulated genes and blue diamonds represent downregulated genes. Lines represent edges. The node size is positively correlated with its degree.

the ribosome assembly process and shares a binding domain of 25S rRNA with P0 in *S. cerevisiae* (28). A study by Kressler *et al* indicated that MRT4 may also function in the assembly of the 60S stalk (29). Based on the above findings, MRT4 may be crucial in rRNA assembly under the restriction of RF59. With the combined biofunctions of RCL1 and MRT4, the increased biosynthesis of rRNA may be the initial response of *C. albicans* to RF59, and this transformation may assist in adapting to RF59 in the short-term exposure environment. The results of the KEGG analysis also showed that the upregulated genes were significantly enriched in the ribosomal and related pathways.

According to the PPI and subnetwork analyses, GIS2 and NOP58 were identified as two hub nodes in the 45-min treatment group. GIS, the DBA-binding zinc-finger protein (30), showed significant upregulation following treatment with RF59 for 45 min. Sammons *et al* reported that GIS2 can be upregulated by limiting glucose, and acts as a translational activator for mRNA within the internal ribosome entry

site in *S. cerevisiae* (31). GIS2 has also been reported to be associated with the initiation and degradation of protein translation (32), and is reported to be key in ribosome biogenesis and cell size control in yeast (33). Taken together, it is hypothesized that GIS2 maybe crucial in ribosome related pathways. KEGG enrichment analysis for the subnetwork of the 45-min treatment group also showed that the involved genes were significantly enriched in the ribosome pathway. NOP58 was significantly upregulated in the 45-min treatment group. As a box C/D snoRNA binding protein, NOP58 and its homologue, NOP56, are required for pre-rRNA splicing and processing (34). NOP58 is also involved in the methylation of 18S rRNA (35). It has been reported that the overexpression of NOP58 in *S. cerevisiae* induces an increased resistance to CANBEF-13, which is a member of a novel family of selective antifungal compounds (35). These results indicate that NOP58 is crucial for rRNA processing and assembly. KEGG analysis for the subnetwork also revealed that the involved genes were significantly enriched in the ribosome pathway. Due to its

Table II. Top 15 nodes identified in topological analysis of the 45-min treated protein-protein interaction network.

| Node	Value
Degree centrality	
RLP7	49
GIS2	44
NOP58	42
RPF2	42
RLP24	41
NOG1	40
URA3	38
MAK5	38
LHP1	37
MAK16	36
NOP4	36
ELP3	36
RRS1	35
RRP1	34
RPA12	33
Betweenness centrality	
URA3	4507.660
GIS2	4461.883
CYR1	2211.606
ELP3	1725.969
ARP8	1309.269
VPS27	1287.934
SAP3	1233.091
RLP7	1129.925
RSD1	1121.732
ADE3	1080.301
HAC1	937.495
GIM3	906.974
RPS0A	886.321
SPT10	881.783
NOP58	806.258
Closeness centrality	
URA3	0.202532
GIS2	0.201835
NOP58	0.199773
ELP3	0.198422
LHP1	0.197753
RLP7	0.196648
CYR1	0.194906
RPF2	0.194690
RPB5	0.194261
MAK5	0.193833
RPC31	0.193407
RRP1	0.193407
RLP24	0.193194
RPA34	0.193194
RPS0A	0.192982
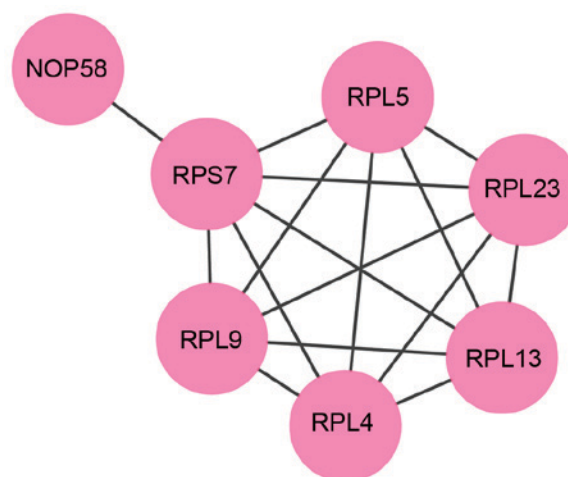
Figure 6. Subnetwork of the protein-protein interaction network of the 45-min RF59 treatment group, vs. control group. Pink circles represent upregulated genes. Lines represent edges. Node size is positively correlated with its degree.

sensitivity to RF-59, NOP58 maybe an important gene for RF-59-resistance.

NOP4 was identified as a shared upregulated protein in the two PPI networks. Sun and Woolford reported that NOP4 is a crucial NOP for the accumulation of 60S ribosomal subunits and pre-RNA processing (36). It has also been reported that NOP4 has four essential RNA recognition motifs, which are necessary for the biogenesis of ribosomes (37). With a deficiency of NOP4, the production of 27S pre-RNAs is normalized but degraded rapidly, and this result may lead to a deficiency of mature 25S rRNA synthesis in yeast (36,38). However, the detailed biofunctions of NOP4 in *C. albicans* remain to be fully elucidated, and further investigation of NOP4 in *C. albicans* is required.

From the *in silico* analysis, it was identified that RF59 can act as an antifungal drug to inhibit the infection of *C. albicans* via affecting ribosomal and related pathways, however, there were limitations to the present study. Although a series of DEGs were found to be important in the drug resistance of *C. albicans*, there remains a lack of experimental verification of these. In addition, the sample size in each group was three, and this maybe not sufficient to draw a valid conclusion. Finally, as drug resistance was found to be the result of long-term drug use, a longer treated duration may be considered.

In conclusion, RF59 maybe a promising therapeutic drug for candidiasis via acting on the ribosome and its associated pathways in *C. albicans*. RCL1, NOP4, MRT4, GIS2 and NOP58 may be important during this procedure. It is essential to further investigate the detailed biofunctions of these genes in *C. albicans* in order to provide novel insights into the pathogenesis of candidiasis and its therapy.

Acknowledgements

References

1. Lara HH, Romero-Urbina DG, Pierce C, Lopez-Ribot JL, Arellano-Jiménez MJ and Jose-Yacaman M: Effect of silver nanoparticles on *Candida albicans* biofilms: An ultrastructural study. J Nanobiotechnology 13: 91, 2015.
2. Yapar N: Epidemiology and risk factors for invasive candidiasis. Ther Clin Risk Manag 10: 95-105, 2014.
3. Kullberg BJ and Arendrup MC: Invasive candidiasis. N Engl J Med 373: 1445-1456, 2015.
4. Odds FC, Brown AJ and Gow NA: Antifungal agents: Mechanisms of action. Trends Microbiol 11: 272-279, 2003.
5. Sharma M, Biswas D, Kotwal A, Thakuria B, Kakati B, Chauhan BS and Patras A: Ibuprofen-mediated reversal of fluconazole resistance in clinical isolates of *Candida*. J Clin Diagn Res 9: DC20-DC22, 2015.
6. Magnuson JG: Potential role of RTA3 and GNP3 transport genes in the quorum sensing response of *Candida albicans*. 2015.
7. Raska M, Běláková J, Krupka M and Weigl E: Candidiasis-Do we need to fight or to tolerate the *Candida* fungus? Folia Microbiol (Praha) 52: 297-312, 2007.
8. Soll DR: The role of phenotypic switching in the basic biology and pathogenesis of *Candida albicans*. J Oral Microbiol 6, 2014.
9. Vandeputte P, Ischer F, Sanglard D and Coste AT: In vivo systematic analysis of *Candida albicans* Zn2-Cys6 transcription factors mutants for mice organ colonization. PLoS One 6: e26962, 2011.
10. Sanglard D, Coste A and Ferrari S: Antifungal drug resistance mechanisms in fungal pathogens from the perspective of transcriptional gene regulation. FEMS Yeast Res 9: 1029-1050, 2009.
11. Gautier L, Cope L, Bolstad BM and Irizarry RA: affy-analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 20: 307-315, 2004.
12. Bolstad BM, Irizarry RA, Astrand M and Speed TP: A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 19: 185-193, 2003.
13. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U and Speed TP: Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 4: 249-264, 2003.
14. Smyth GK: LIMMA: Linear models for microarray data. In: Bioinformatics and Computational Biology Solutions Using R and Bioconductor. Gentleman R, Carey V, Huber W, Irizarry R and Dudoit S (eds). Springer-Verlag New York, New York, NY, pp397-420, 2005.
15. Huang DW, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, Stephens R, Baseler MW, Lane HC and Lempicki RA: The DAVID gene functional classification tool: A novel biological module-centric algorithm to functionally analyze large gene lists. Genome Biol 8: R183, 2007.
16. Kanehisa M and Goto S: KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28: 27-30, 2000.
17. Von Mering C, Huynen M, Jaeggi D, Schmidt S, Bork P and Snel B: STRING: A database of predicted functional associations between proteins. Nucleic Acids Res 31: 258-261, 2003.
18. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B and Ideker T: Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res 13: 2498-2504, 2003.
19. Tang Y, Li M, Wang J, Pan Y and Wu FX: CytoNCA: A cytoscape plugin for centrality analysis and evaluation of protein interaction networks. Biosystems 127: 67-72, 2015.
20. He X and Zhang J: Why do hubs tend to be essential in protein networks? PLoS Genet 2: e88, 2006.
21. Wu G, Dawson E, Duong A, Haw R and Stein L: ReactomeFIViz: A Cytoscape app for pathway and network-based data analysis. Version 2. F1000Res 3: 146, 2014.
22. Genschik P, Billy E, Swianiewicz M and Filipowicz W: The human RNA 3'-terminal phosphate cyclase is a member of a new family of proteins conserved in Eucarya, Bacteria and Archaea. EMBO J 16: 2955-2967, 1997.
23. Horn DM, Mason SL and Karbstein K: Rcl1 protein, a novel nuclease for 18 S ribosomal RNA production. J Biol Chem 286: 34082-34087, 2011.
24. Delprato A, Al Kadri Y, Pérébaskine N, Monfoulet C, Henry Y, Henras AK and Fribourg S: Crucial role of the Rcl1p-Bms1p interaction for yeast pre-ribosomal RNA processing. Nucleic Acids Res 42: 10161-10172, 2014.
25. Enjalbert B, Smith DA, Cornell MJ, Alam I, Nicholls S, Brown AJ and Quinn J: Role of the Hog1 stress-activated protein kinase in the global transcriptional response to stress in the fungal pathogen *Candida albicans*. Mol Biol Cell 17: 1018-1032, 2006.
26. Huh WK, Falvo JV, Gerke LC, Carroll AS, Howson RW, Weissman JS and O'Shea EK: Global analysis of protein localization in budding yeast. Nature 425: 686-691, 2003.
27. Rodríguez-Mateos M, Abia D, García-Gómez JJ, Morreale A, de la Cruz J, Santos C, Remacha M and Ballesta JP: The amino terminal domain from Mrt4 protein can functionally replace the RNA binding domain of the ribosomal P0 protein. Nucleic Acids Res 37: 3514-3521, 2009.
28. Rodríguez-Mateos M, García-Gómez JJ, Francisco-Velilla R, Remacha M, de la Cruz J and Ballesta JP: Role and dynamics of the ribosomal protein P0 and its related trans-acting factor Mrt4 during ribosome assembly in *Saccharomyces cerevisiae*. Nucleic Acids Res 37: 7519-7532, 2009.
29. Kressler D, Hurt E, Bergler H and Bassler J: The power of AAA-ATPases on the road of pre-60S ribosome maturation-molecular machines that strip pre-ribosomal particles. Biochim Biophys Acta 1823: 92-100, 2012.
30. Jang Y, Lee H, Lee SW, Choi YS, Ahn BJ, Kim GH and Kim JJ: Cu(II)-induced molecular and physiological responses in the brown-rot basidiomycete Polyporales sp. KUC9061. J Appl Microbiol 113: 790-797, 2012.
31. Sammons MA, Samir P and Link AJ: *Saccharomyces cerevisiae* Gis2 interacts with the translation machinery and is orthogonal to myotonic dystrophy type 2 protein ZNF9. Biochem Biophys Res Commun 406: 13-19, 2011.
32. Matia-González AM and Gerber AP: Approaches for dissecting RNA-binding protein networks. Fungal RNA Biology, pp347-370, 2014.
33. Scherrer T, Femmer C, Schiess R, Aebersold R and Gerber AP: Defining potentially conserved RNA regulons of homologous zinc-finger RNA-binding proteins. Genome Biol 12: R3, 2011.
34. Anderson I, Göker M, Nolan M, Lucas S, Hammon N, Deshpande S, Cheng JF, Tapia R, Han C, Goodwin L, *et al*: Complete genome sequence of the hyperthermophilic chemolithoautotroph *Pyrolobus fumarii* type strain (1A). Stand Genomic Sci 4: 381-392, 2011.
35. Mircus G, Albert N, Ben-Yaakov D, Chikvashvili D, Shadkchan Y, Kontoyiannis DP and Osherov N: Identification and characterization of a novel family of selective antifungal compounds (CANBEFs) that interfere with fungal protein synthesis. Antimicrob Agents Chemother 59: 5631-5640, 2015.
36. Sun C and Woolford JL Jr: The yeast NOP4 gene product is an essential nucleolar protein required for pre-rRNA processing and accumulation of 60S ribosomal subunits. EMBO J 13: 3127-3135, 1994.
37. Sun C and Woolford JL Jr: The yeast nucleolar protein Nop4p contains four RNA recognition motifs necessary for ribosome biogenesis. J Biol Chem 272: 25345-25352, 1997.
38. Harnpicharnchai P, Jakovljevic J, Horsey E, Miles T, Roman J, Rout M, Meagher D, Imai B, Guo Y, Brame CJ, *et al*: Composition and functional characterization of yeast 66S ribosome assembly intermediates. Mol Cell 8: 505-515, 2001.