Candida albicans triggers the expression of inflammatory genes in human umbilical vein endothelial cells

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Abstract. The aim of the present study was to investigate the pathogenesis of *Candida albicans* in human umbilical vein endothelial cells (HUVECs) and to screen for aberrantly expressed genes during the process of infection. GSE7355 (accession no.) was downloaded from the National Center of Biotechnology Information Gene Expression Omnibus database and used to identify the differentially-expressed genes (DEGs) between the two groups, which included 4 samples from an untreated HUVEC control group, and 4 samples from HUVECs exposed to *C. albicans*. Subsequently, the gene ontology (GO) function package was used to perform GO and pathway enrichment analysis, prior to the extraction of DEG correlations in the Kyoto Encyclopedia of Genes and Genomes. A protein-protein interaction (PPI) network was constructed using the String database. In total, 77 DEGs were identified, including 69 upregulated and 8 downregulated DEGs in the *C. albicans*-infected HUVEC samples. DEGs were significantly enriched in response to external stimuli and chemokine activity. In addition, DEG FBJ murine osteosarcoma viral oncogene homolog (FOS) and interleukin (IL)-6 were significantly enriched in the Toll-like receptor signaling pathway. Nuclear factor κ light polypeptide gene enhancer in B cells 2 (NFKB2) was significantly enriched in the mitogen-activated protein kinase signaling pathway. In the interaction network of DEGs, according data included in the KEGG database, FOS and NFKB2 had higher connectivity degrees. Notably, FOS, IL-6 and intercellular adhesion molecule 1 were demonstrated to have higher connectivity degrees in the PPI network. FOS, IL-6 and NFKB2 may be important genes for *C. albicans* infection in HUVECs, and these genes may act as therapeutic targets to treat patients infected with *C. albicans*.

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Introduction

The Candida species causes nosocomial bloodstream infections under certain circumstances (1). *Candida albicans* is a type of Candida species that may act as an opportunistic pathogen in immunocompromised or immunosuppressed patients (2). The incidence of candidemia is 1.1-24 cases/100,000 individuals, and the associated mortality is >30%, even when patients are treated with antifungal agents (1,3,4). Therefore, elucidating the molecular mechanism underlying *C. albicans* infection and developing novel clinical approaches is urgently required.

In recent years, the mechanism underlying *C. albicans* infection has been clarified. Sun *et al* (5) demonstrated that *Ssa1* plays a key role in the ability of *C. albicans* to damage host cells via binding to host endothelial cell cadherins and inducing host cell endocytosis in the models of oropharyngeal candidiasis. Furthermore, a previous study demonstrated that endothelial cells respond to infection with *C. albicans* by synthesizing interleukin (IL)-8 *in vitro* (6). Müller *et al* (7) suggested that activation of the p38 mitogen-activated protein kinase (MAPK) cascade is important for *Candida*-induced expression of chemokine (C-X-C Motif) ligand 8/IL-8 in endothelial cells. Several studies have demonstrated that the pathogenicity of *C. albicans* is induced by hyphae and epithelial cell damage (8,9). Notably, Moyes *et al* (10) demonstrated that the MAPK/MKP1/c-Fos signaling pathway is important for the formation of *C. albicans* hyphae in oral epithelial cells. However, the molecular mechanism underlying the host immune response and pathogen recognition is complex, and therefore our understanding of *C. albicans* infection is not fully complete.

Gene expression microarray analysis is used to observe changes in gene expression levels in various types of disease (11,12). Müller *et al* (7) provided the microarray data of GSE7355 (accession no.), and analyzed the differentially-expressed genes (DEGs) of human umbilical

vein endothelial cells (HUVECs) following exposure to *C. albicans*. In addition, they investigated the nuclear factor (NF)-κB and p38 MAPK signaling pathways in *C. albicans* infection. However, the interaction between DEGs was not analyzed, and a protein-protein interaction (PPI) network was not constructed.

To fully understand the HUVEC response to *C. albicans*, in the present study the microarray profile of HUVECs infected with *C. albicans* were analyzed and compared to a control. The DEGs between the two groups were screened, and a gene ontology (GO) function package was used to perform GO and pathway enrichment analysis of the DEGs. The extraction of the correlations among the DEGs were then carried out using the Kyoto Encyclopedia of Genes and Genomes (KEGG). Finally, a PPI network was constructed.

Materials and methods

Analysis of microarray data. The gene expression data GSE7355 (7) was downloaded from the National Center of Biotechnology Information Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) using the GPL96 platform of Affymetrix Human Genome U133A Arrays. A total of 8 samples were used in the present study, including 4 samples from untreated HUVEC monolayers (GSM177134, GSM177140, GSM17177141 and GSE177142) that served as the control group, and 4 samples from HUVECs exposed to *C. albicans* (GSM177136, GSM177137, GSM17177138 and GSE177139) that served as the experimental group. Raw data were downloaded for further analysis.

Data preprocessing and identification of DEGs. The Affy package (http://www.bioconductor.org/packages/release/bioc/html/affy.html) (13) of Bioconductor (http://bioconductor.org/) was used to calculate the gene expression levels. Subsequently, a robust multiarray average algorithm (13) was used to perform the quartile data normalization. A t-test was carried out using the Limma package (http://www.bioconductor.org/packages/release/bioc/html/limma.html) (14) and applied to screen for DEGs between the two groups. $P<0.05$ and $|\log FC|>0.58$ were selected as the criterion for DEGs.

GO and pathway enrichment analysis. Frequently, GO is used to conduct the functional enrichment analysis for large-scale genes (15). To identify the functions of the DEGs between the control and experimental samples, GO enrichment analysis was performed. In addition, KEGG pathway enrichment analysis was carried out for the DEGs, and bioinformatics databases containing all types of biochemistry signaling pathways were assessed (16). The GOFunction package (http://www.bioconductor.org/packages/release/bioc/html/GOFunction.html) of Bioconductor was used to perform the GO and pathway enrichment analysis. A $P<0.05$ and gene counts ≥2 were considered as the cut-off value. Furthermore, the correlation among DEGs was extracted according to the interactions of the genes in the KEGG.

Construction of a protein-protein interaction (PPI) network. The Search Tool for the Retrieval of Interacting Genes (STRING) database (http://string-db.org/) (17) is an online database that provides information on the interaction between proteins. In the present study, the STRING database was used to screen functional interactions between DEGs. A combined score >4 were regarded as the threshold. According to the criterion, Cytoscape (http://cytoscapeweb.cytoscape.org/) (18) was then used to display the PPI network.

Results

Identification of DEGs. Compared with the untreated HUVEC samples, a total of 77 DEGs were identified, including 69 upregulated DEGs corresponding to 187 transcripts, and 8 downregulated DEGs corresponding to 16 transcripts in the candida-infected HUVEC samples. The cluster heat map of 77 DEGs is presented in Fig. 1.

GO and pathway enrichment analysis. The GOfunction package was used to identify GO functions and signaling pathways for the significant DEGs. Notably, DEGs from the *Candida*-infected HUVEC samples were significantly enriched in response to external biological process stimuli (P<1.00E-15), chemokine activity (P=5.58E-08) and cytokine activity of molecular function (P=4.17E-07; Table I). In addition, DEGs were significantly enriched in the nodulation-like receptor signaling pathway (P=1.82E-08), toll-like receptor signaling pathway (P=1.00E-02) and MAPK signaling pathway (P=2.30E-02). The results of the pathway enrichment analysis are displayed in Table II.

The interaction network between the DEGs extracted from the KEGG, including 12 nodes and 10 edges, is shown in Fig. 2. In this network, FBJ murine osteosarcoma viral oncogene homolog (FOS) and nuclear factor κ light polypeptide gene enhancer in B cells 2 (NFKB2; p49/p100) had the highest connectivity degrees.

Construction of the PPI network. In the present study, 52 nodes and 226 edges were used to construct the PPI network (Fig. 3). Notably, several nodes exhibited higher connectivity degrees: FOS (degree, 30), IL-6 (degree, 26), intercellular adhesion molecule 1 (degree, 22) and prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase; degree, 21).

Discussion

The present study examined the gene expression data GSE7355 and investigated the HUVEC reaction patterns to the fungal pathogen *C. albicans*. In total, 77 DEGs were identified, including 69 upregulated DEGs corresponding to 187 transcripts and 8 downregulated DEGs corresponding to 16 transcripts in *C. albicans*-infected HUVEC samples. Notably, DEGs such as FOS and IL-6 were significantly enriched in the toll-like receptor signaling pathway, whereas NFKB2 was significantly enriched in the MAPK signaling pathway. In addition, FOS and IL-6 exhibited high connectivity degrees in the PPI network.

Toll-like receptors are the primary innate recognition system for microbial invaders in vertebrates, and are responsible for the immune response to microbial pathogens (19). Accumulating evidence suggests that the activation of toll-like

Table I. Gene ontology functional enrichment analysis of DEGs.

Gene ontology	Function	Total genes (n)	Enriched DEGs (n)	P-value
BP	Response to external stimulus	1,941	41	1.00E-15
	Response to stress	3,341	45	7.33E-15
	Cell migration	1,015	26	5.02E-14
	Response to stimulus	7,662	63	2.77E-13
	Cell motility	1,095	26	2.94E-13
CC	Extracellular space	1,212	19	2.67E-07
	I-κB/NF-κB complex	5	3	6.61E-07
	Bcl-3/NF-κB2 complex	2	2	1.66E-05
	Side of membrane	300	8	3.10E-05
	Cell surface	645	10	2.90E-04
MF	Protein binding	8,384	61	5.89E-09
	Chemokine activity	46	6	5.58E-08
	Chemokine receptor binding	56	6	1.87E-07
	Cytokine activity	210	9	4.17E-07
	Binding	12,580	70	2.53E-06

DEG, differentially-expressed gene; BP, biological process; CC, cellular components; MF, molecular function.; I-κB, inhibitor of κB; NF-κB, nuclear factor κB; Bcl-3, B cell lymphoma 3.

Table II. KEGG signaling pathway analysis of DEGs.

Name	Total genes (n)	Enriched DEGs (n)	P-value
NOD-like receptor signaling pathway	58	8	1.82E-08
Rheumatoid arthritis	91	8	6.56E-07
Malaria	51	6	3.35E-06
Cytokine-cytokine receptor interaction	265	11	8.15E-06
Osteoclast differentiation	128	7	7.98E-05
African trypanosomiasis	35	4	1.86E-04
Pathways in cancer	326	10	2.87E-04
Chemokine signaling pathway	189	7	0.000877
Chagas disease (American trypanosomiasis)	104	5	0.001629
Epithelial cell signaling in *Helicobacter pylori* infection	68	4	0.00236
Toll-like receptor signaling pathway	102	4	0.010011
Amoebiasis	106	4	0.011421
RIG-I-like receptor signaling pathway	71	3	0.021015
Leishmaniasis	72	3	0.021802
MAPK signaling pathway	268	6	0.023041
B cell receptor signaling pathway	75	3	0.02426
Small cell lung cancer	85	3	0.033497
Bladder cancer	42	2	0.047575

KEGG, Kyoto Encyclopedia of Genes and Genomes; DEG, differentially-expressed genes; NOD, nodulation; RIG, retinoid acid-inducible gene; MAPK, mitogen-activated protein kinase.

receptors is important for *Candida* infection (20,21). Notably, Zakikhany *et al* (22) suggested that hypha formation was crucial for the pathogenicity of *C. albicans* as well as the proinflammatory responses of mucosal tissues, which protect against subsequent fungal infection mediated by toll-like receptor 4 signaling (23). In the current study, FOS and IL-6 were significantly enriched in the toll-like receptor signaling pathway. FOS activation is mediated by hypha-associated surface moieties including MKP1 (24). Previously, a study demonstrated that hypha formation is dependent on the MAPK

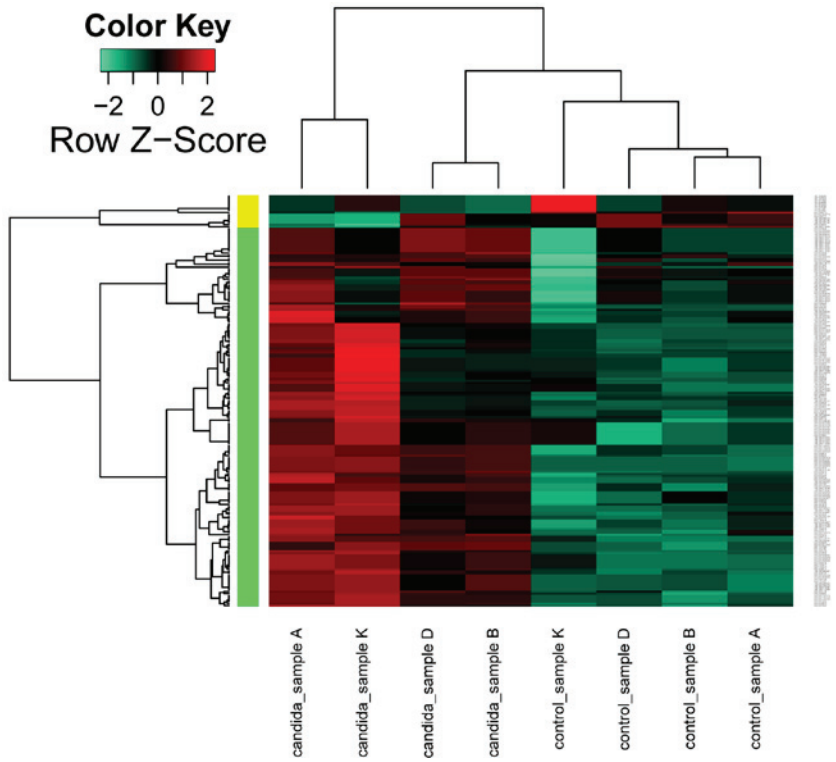

Figure 1. Cluster heat map of the 77 differentially expressed genes. The color represents the levels of gene expression in the 8 samples. The horizontal axis represents the samples, and the vertical axis represents the differentially expressed genes. Genes with high expression levels are presented in red; genes with low expression levels are presented in green.

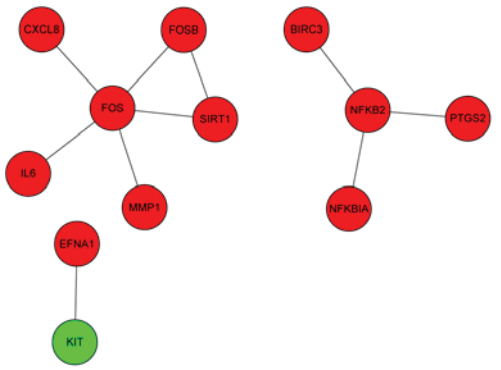

Figure 2. Interaction network of differentially expressed genes. Genes that are upregulated are presented in red; genes that are downregulated are presented in green.

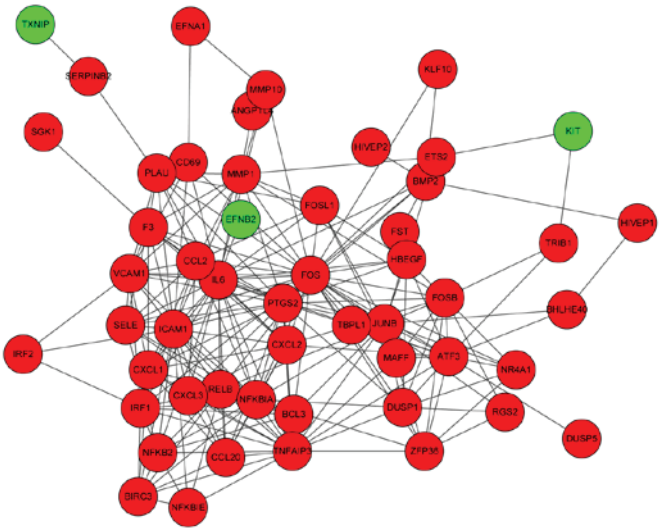

Figure 3. Protein-protein interaction network of differentially expressed genes. Genes that are upregulated are presented in red; genes that are downregulated are presented in green.

response, constituted by the activation of FOS and MKP1 (8). Moyes *et al* (10) reported that the MAPK/MKP1/FOS signaling pathway is important for the formation of *C. albicans* hyphae in oral epithelial cells. Furthermore, the results of the present study demonstrated that FOS was upregulated, results which were concordant with those of Moyes *et al* (25) who demonstrated that FOS expression levels gradually increased with the progression of *C. albicans* infection in vaginal epithelial cells. In addition, hypha formation dependent on FOS activation and cell damage can induce the production of cytokines (10). IL-6 has been reported to protect against *Candida* infection (26). The present study demonstrated that IL-6 expression levels were upregulated, results which were concordant with those of Mostefaoui *et al* (27). Mostefaoui *et al* (27) demonstrated that IL-6 mRNA expression levels were significantly upregulated in human oral mucosa tissue following infection with *C. albicans*. These findings demonstrate that FOS and IL-6 may have important roles in *C. albicans* infection via the toll-like receptor signaling pathway.

Infection of epithelial cells with *C. albicans* causes the activation of NF-κB, as well as a MAPK signaling response, which further induces a pro-inflammatory response (28). Furthermore, a previous study suggested that MAPK signaling

pathways may served as targets for antifungal therapy (29). Notably, another investigation demonstrated that the MAPK signaling pathway enables human epithelial tissues to regulate innate immune responses against the hyphae of *C. albicans* (25). In the present study, NFKB2 expression levels were demonstrated to be upregulated and significantly enriched in the MAPK signaling pathway. NFKB2 is a member of the NF-κB signal transduction pathway which has important roles in inflammatory and immune responses (30). Furthermore, fungal infection may be responsible for the release of chemotactic molecules in innate immune effector cells (31). Therefore, these data suggested that NFKB2 may have important roles in *C. albicans* infection via the MAPK signaling pathway.

In conclusion, the results of the present study further elucidated the mechanism underlying the effects of *C. albicans* infection in HUVECs. The screened DEGs, including FOS, IL-6 and NFKB2 may be important genes for the pathogenesis of *C. albicans* infection in HUVECs, and these genes may serve as therapeutic targets to treat patients infected with *C. albicans*. However, this study presented some limitations. The most important limitation was that the study was conducted using bioinformatics methods, but the results have not been further demonstrated through experiments. Therefore, further investigation using animal experiments should be considered.

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