Original Article
CXCL5 is increased in *Candida albicans* infected patients and human pulmonary epithelial cells

Wei Chen[1], Xiaoshu Zuo[1], Lu Wang[1], Tielong Chen[2]

[1]Department of Intensive Care Unit, Renmin Hospital of Wuhan University, Wuhan, P. R. China; [2]Department of Infectious Diseases, Zhongnan Hospital of Wuhan University, Wuhan, P. R. China

Abstract: CXCL5 is critical for recruiting neutrophils and its aberrant production in lung epithelial cells has been confirmed in various mouse models of bacterial infectious diseases. The purpose of this study was to determine whether *Candida albicans* (*C. albicans*) upregulates CXCL5 expression of human lung epithelial cells. Here, we demonstrate that serum levels of CXCL5 are significantly increased and positively correlated with serum $(1\rightarrow3)$-β-D-glucan (BG) levels in HIV patients with pulmonary candidiasis compared with patients with no evidence of *Candida* coinfection. Furthermore, serum CXCL5 levels were markedly decreased in patients after treatment with fluconazole for two weeks and positive correlation between serum CXCL5 and BG levels was caused by *C. albicans* infection. In *in vitro* cell culture, we further show that *C. albicans* significantly upregulate CXCL5 expression in both human lung epithelial cell line A549 and bronchial epithelial cell line BEAS-2B by activating PI3K, p38 MAPK, and NF-κB pathways. Together, our study provides novel insight into regulation of CXCL5 chemokine production of lung epithelial cells during *C. albicans* infection.

Keywords: *Candida albicans*, CXCL5, pulmonary epithelial cells, pulmonary candidiasis

Introduction

Pulmonary candidiasis occupies up to 86% of fungal pneumonia in HIV-infected individuals or immunosuppressed critically-ill patients [1, 2]. Among causative agents, the most common is Candida albicans (C. albicans), which was isolated from 60-70% of all samples every year [3, 4]. As the first line of defense against C. albicans infection, epithelial cells play a key role in signaling immune cells and induction of innate immune responses [5, 6]. Therefore, it is essential to evaluate the effects of *C. albicans* on pulmonary epithelial cells for a better understanding of *C. albicans* pathogenesis during pulmonary candidiasis.

The process of inflammatory cell recruitment toward the lungs in response to infection is initiated by the release of proinflammatory cytokines and a variety of chemokines like CCL2 (MCP-1), CCL5 (RANTES), CCL3/4 (MIP-1αβ), CXCL10 (IFN-inducible protein 10), and CXCL8 (IL-8) from infected epithelial cells [7]. CXCL1, -2, -3, -5, and -8 proteins are neutrophil-activat-ing chemokines contributing to establishment of a chemotactic gradient during inflammation [8]. CXCL5/ENA-78 (epithelial neutrophil-acti-vating peptide 78) mediated CXCR1/CXCR2-dependent neutrophil recruitment has been observed in bacterial, fungal, and viral infec-tions [9-12]. Type I and type II alveolar epithelial cell-secreted CXCL5 plays an important role in LPS (lipopolysaccharide)-induced lung inflam-mation and is critical for polymorphonuclear leukocytes (PMN)-driven destructive inflamma-tion in pulmonary tuberculosis [13-16]. In addi-tion, CXCR1 and CXCL5 were highly induced in a *Candida*-infected kidney in a mouse model of systemic candidiasis and CXCR1-dependent neutrophil effector function has been shown to be a critical innate protective mechanism of fungal clearance and host survival [10, 12]. However, the effect of *C. albicans* on CXCL5 expression of pulmonary epithelial cells during pulmonary candidiasis remains poorly defined.

In this study, we demonstrated that serum lev-els of CXCL5 were significantly increased and positively correlated with serum $(1\rightarrow3)$-β-D-

glucan (BG) levels in HIV patients with pulmonary candidiasis compared with patients without pulmonary candidiasis. Furthermore, after treatment with Fluconazole for two weeks, serum CXCL5 levels were markedly decreased. We further showed that *C. albicans* upregulated CXCL5 expression in both human lung epithelial cells and bronchial epithelial cells by activating PI3K, p38 MAPK, and NF-κB pathways, while inhibitors of JNK and MEK1/2 pathways had no obvious effect on *C. albicans*-induced CXCL5 secretion. Thus, for the first time, our study describes the effect of *C. albicans* on CXCL5 chemokine production of lung epithelial cells.

Materials and methods

Reagents

JNK inhibitor (SP600125), MEK1/2 inhibitor (U0126), PI3K inhibitor (LY294002), p38 MAPK inhibitor (SB203580), NF-κB inhibitor (BAY 11-7082), and proteasome inhibitor (MG-132) were purchased from Beyotime Institute of Biotechnology (Beyotime, Shanghai, China).

Participants

This study was approved by the Ethics Committee of Renmin Hospital of Wuhan University, Wuhan, China. Written informed consent was obtained from all participants. Serum samples of 48 HIV-1-infected patients, with no evidence of *Candida* coinfection, and 48 HIV-1-infected patients with pulmonary candidiasis (including 29 *Candida albicans*, 9 *Candida tropicalis*, 7 *Candida glabrata,* and 3 *Candida krusei*) were collected in the Center for Disease Control and Prevention, Wuhan, China, from 2014 to 2016. All patients were previously identified as HIV-1-seropositive by commercial ELISA test and Western blotting (MP Diagnostics TM HIV BLOT 2.2, MP Biomedicals, Singapore), according to National Guideline for Detection of HIV/AIDS in China (edited by Chinese Center for Disease Control and Prevention), as previously described [17]. Cancer patients with HIV infection and HIV patients with tuberculosis co-infection were excluded. Baseline and clinical characteristics of HIV patients are shown in **Table 1**. All HIV-infected patients received antiretroviral treatment. BG levels were measured with the Glucatell kit (Glucatell; Associates of Cape Cod, Falmouth, MA, USA), according to manufacturer recommendations. The cutoff value was 80 pg/mL.

Cell culturing

Human lung epithelial A549 cells were maintained in complete Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS) (HyClone, CA, USA) and supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin (100×, Beyotime, shanghai, China). Human bronchial epithelial BEAS-2B cells were cultured in Clonetics Bronchial Epithelial Growth Medium (BEGM) (Lonza, CA, USA). Cells were cultured at 37°C in a humidified 5% CO_2 atmosphere.

Preparation of C. albicans

A lyophilized strain of *C. albicans* ATCC10231 was obtained from China Center for Type Culture Collection (CCTCC, Wuhan, China). Routine cultivation was performed on SDA culture medium and incubated at 35°C for 48 hours [2]. *C. albicans* were isolated, counted, and then washed with PBS by centrifuging at 5000× g for 10 minutes.

Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated with TRIzol Reagent (Life Technologies, CA, USA), according to manufacturer protocol. First-strand cDNA was synthesized from total RNA using ReverTra Ace-α First-strand cDNA Synthesis Kit (Toyobo, Osaka, Japan). Relative mRNA expression of CXCL5 was performed using real-time RT-PCR with SYBR Green real-time PCR Master Mix kit (Toyobo, Osaka, Japan) and determined using StepOnePlus Real-Time PCR system (Applied Biosystems). Relative quantities of mRNAs were obtained using the $2^{-\Delta\Delta Ct}$ method. Human CXCL5 primer sequences were as follows: 5'-GTGGTAGCCTCCCTGAAGAAC-3', 5'-TCCTTGTTTCCACCGTCCAA-3. GAPDH was used as the endogenous control [18].

Determination of CXCL5 levels

CXCL5 levels in serum and culture supernatants were determined by Human CXCL5 ELISA MAX™ Deluxe set (Biolegend, CA, USA), in accordance with manufacturer instructions.

Western blotting

Human lung epithelial A549 cells and bronchial epithelial BEAS-2B cells were washed twice with cold PBS and lysed using RIPA buffer (Beyotime, Shanghai, China). The same amount of each sample was subjected to and electro-

Table 1. Baseline and clinical characteristics of HIV patients

	PC	Non-PC	χ^2	*p* value
Number	48	48		
Age (%)			0.8715	*0.6488*
≤20	2 (4.17)	4 (8.33)		
>20 and ≤60	41 (85.42)	38 (79.17)		
>60	5 (10.41)	6 (12.50)		
Sex (male/female)	35/12	32/15	0.4447	*0.5049*
Marital status (%)			0.4149	*0.9731*
Married	33 (68.75)	34 (70.83)		
Unmarried	11 (22.91)	9 (18.75)		
Divorced	2 (4.17)	2 (4.17)		
Widowed	2 (4.17)	3 (6.25)		
Profession (%)			0.2810	*0.9636*
Employed	33 (68.75)	35 (72.92)		
Unemployed	10 (20.83)	8 (16.67)		
Students	2 (4.17)	2 (4.17)		
Retired	3 (6.25)	3 (6.25)		
Route of infection (%)			2.3210	*0.5086*
Sextual contact	34 (70.83)	30 (62.50)		
Blood transmission	6 (12.50)	11 (22.92)		
Mother-to-child transmission	2 (4.17)	3 (6.25)		
Unknown	6 (12.50)	4 (8.33)		
CD4$^+$ T cell number per μl (%)			1.5240	*0.4668*
≤200	33 (68.75)	30 (62.50)		
>200 and ≤500	11 (22.92)	10 (20.83)		
>500	4 (8.33)	8 (16.67)		
Neutrophil number (10^9/L)	5.17 ± 2.08	3.23 ± 0.79		*0.0193*

phoresed by SDS-PAGE gel, followed by transferring to PVDF membranes (Millipore, USA). The membranes were blocked in 5% non-fat milk overnight and then incubated with CXCL5 primary antibody (1:500 dilution, Novus Biologicals, USA) [19]. HRP-conjugated IgG antibodies (Santa Cruz, USA) were used as the secondary antibody. GAPDH was used as a control (Abcam, USA). Signals were detected using ECL detection reagent (Millipore, USA), following manufacturer instructions.

Statistical analysis

Data were analyzed by Graphpad Prism V.5.00 software (GraphPad Software, CA, USA). Count data of HIV patients were analyzed using χ^2 test. Comparison between two groups for statistical significance was performed with unpaired or paired Student's t-test. For more groups, one-way ANOVA followed by Neuman-Keuls post hoc test was used. P<0.05 was considered statistically significant. Spearman's

rank test was used to assess association between BG and CXCL5 levels. In addition, multivariate linear regression with enter and backward method was performed on SPSS 17.0 (SPSS Inc. Chicago, IL, USA) to adjust for other covariates (age, sex, CD4$^+$ T cell number, and neutrophil number). CXCL5 was set as dependent variable.

Results

Serum CXCL5 is elevated in patients with pulmonary candidiasis

Ninety-six HIV patients were divided into two groups with or without pulmonary candidiasis (**Table 1**). Neutrophil number in peripheral blood was significantly higher in HIV patients with pulmonary candidiasis compared to patients with no evidence of *Candida* coinfection (5.17 ± 2.08 *vs.* 3.23 ± 0.79, *P=0.0193<0.05*). Then, serum levels of BG and CXCL5 were determined before antifungal therapy. Our results showed that serum BG (255.30 ± 97.93 *vs.* 54.09 ± 36.30, pg/ml, **Figure 1A**) and CXCL5 (1.77 ± 0.28 *vs.* 0.77 ± 0.21, ng/ml, **Figure 1B**) levels were significantly increased in HIV patients with pulmonary candidiasis compared to patients with no evidence of *Candida* coinfection. Correlation analysis further revealed that serum CXCL5 protein levels were positively correlated with BG levels in HIV patients with pulmonary candidiasis (**Figure 1C**, r^2=0.6371, *P<0.0001*) but not in patients with no evidence of *Candida* coinfection (**Figure 1D**, r^2=0.0131, *P>0.05*). In HIV patients with pulmonary candidiasis, multivariable linear regression analysis further confirmed that CXCL5 was independently positively correlated with neutrophil number (t=3.591, *P=0.001*) and BG levels (t=2.881, *P=0.006*) but no other variables such as age, sex, and CD4$^+$ T cell number (**Table 2**). In addition, serum
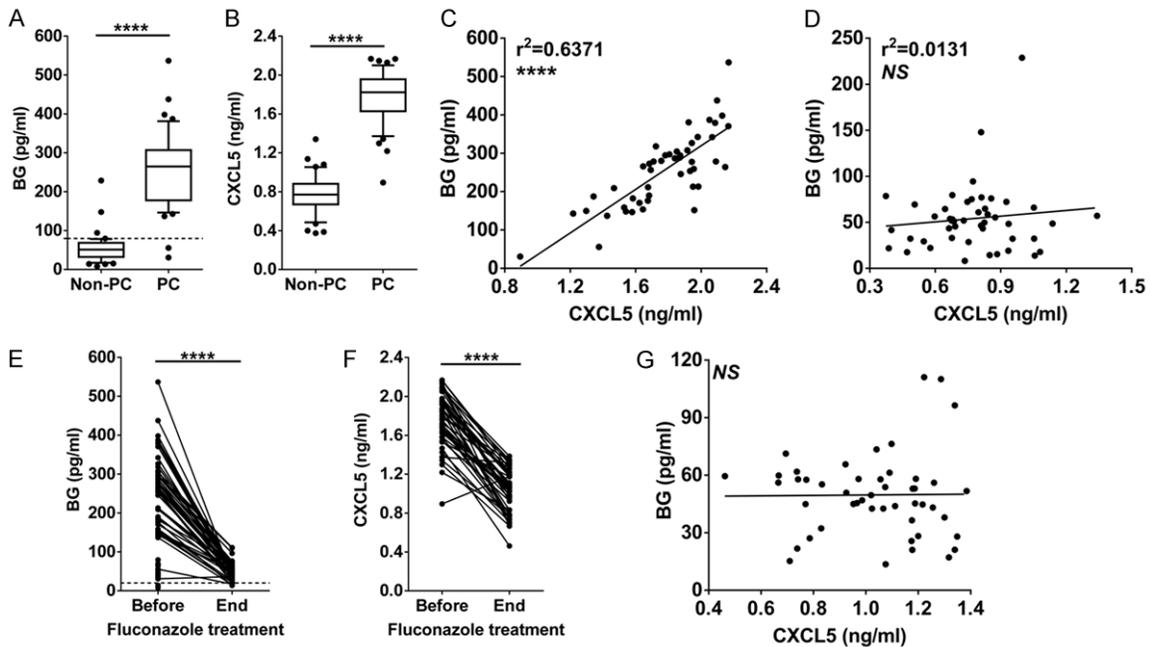
Figure 1. Serum CXCL5 levels were elevated and positively correlated with BG levels in HIV patients with pulmonary candidiasis. (A) Serum BG and (B) CXCL5 levels were detected in 48 HIV patients with pulmonary candidiasis (PC) and 48 HIV patients no evidence of *Candida* coinfection (non-PC). Data were presented as median (P_{10}~P_{90}) and statistically difference was analyzed using unpaired student's *t* test. *****P<0.0001*. (C) Spearman's rank test was used to assess the association of between BG and CXCL5 levels in 48 HIV patients with pulmonary candidiasis. *****P<0.0001*. (D) Spearman's rank test was used to assess the association of between BG and CXCL5 levels in 48 HIV patients no evidence of *Candida* coinfection. (E) Serum BG and (F) CXCL5 levels were detected in HIV patients with pulmonary candidiasis before and after treated with Fluconazole for two weeks (end). Data are presented as median (P_{10}~P_{90}) and statistically difference was analyzed using paired student's *t* test. *****P<0.0001*. (G) Spearman's rank test was used to assess the association of between BG and CXCL5 levels in HIV patients with PC after treated with Fluconzaole. *NS, no significant difference.*

**Table 2.** Multivariate analysis of correlation between BG and CXCL5 levels (multivariable linear regression model)

Variables	t value	p value
Age (years)	-1.662	0.1040
Sex (male/female)	-1.082	0.2856
CD4+ T cell number per μl	-1.461	0.1514
Neutrophil number (10^9/L)	3.591	0.0009*
BG (pg/ml)	2.881	0.0062*

*P<0.05 means statistically significant.

levels of BG (**Figure 1E**) and CXCL5 (**Figure 1F**) were significantly decreased in 48 patients with pulmonary candidiasis after two weeks of fluconazole treatment. Positive correlation between BG and CXCL5 disappeared after fluconazole treatment (**Figure 1G**, *P>0.05*), which is the first-line therapeutic drug of localized and systemic *C. albicans* infection [20, 21]. Thus, positive correlation between serum CXCL5 and BG levels was caused by *C. albicans* infection.

C. albicans induces CXCL5 expression in lung and bronchial epithelial cells

CXCL5-secreting pulmonary epithelial cells is largely responsible for pulmonary PMN recruitment via CXCR2 [13] and, importantly, neutrophils are the major cellular mediators of innate immune response against systemic candidiasis [10]. Thus, we speculated that *Candida* colonized in lung tissues may have the ability to induce CXCL5 expression of lung epithelial cells. Effects of *C. albicans* on CXCL5 expression was evaluated in human bronchial epithelial BEAS-2B cells and human lung epithelial A549 cells. Using qRT-PCR analyses, we first demonstrated that *C. albicans* significantly increased CXCL5 mRNA expression at a multiplicity of infection (MOI) of 1 in both BEAS-2B cells (**Figure 2A**) and A549 cells (**Figure 2D**), in a time-dependent manner, with expression peaking after 120 minutes. We, then, further confirmed that *C. albicans* induced CXCL5 expression in a dose-dependent manner in both BEAS-
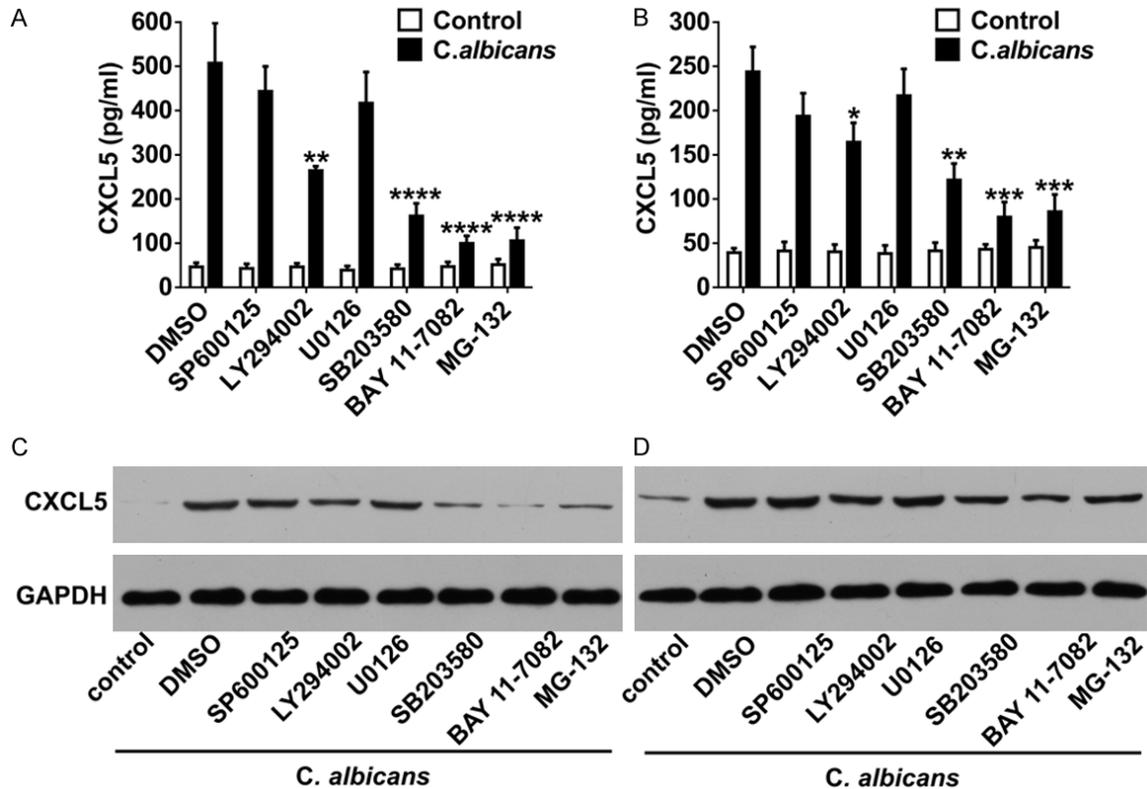
Human bronchial epithelial BEAS-2B cells

Human lung epithelial A549 cells
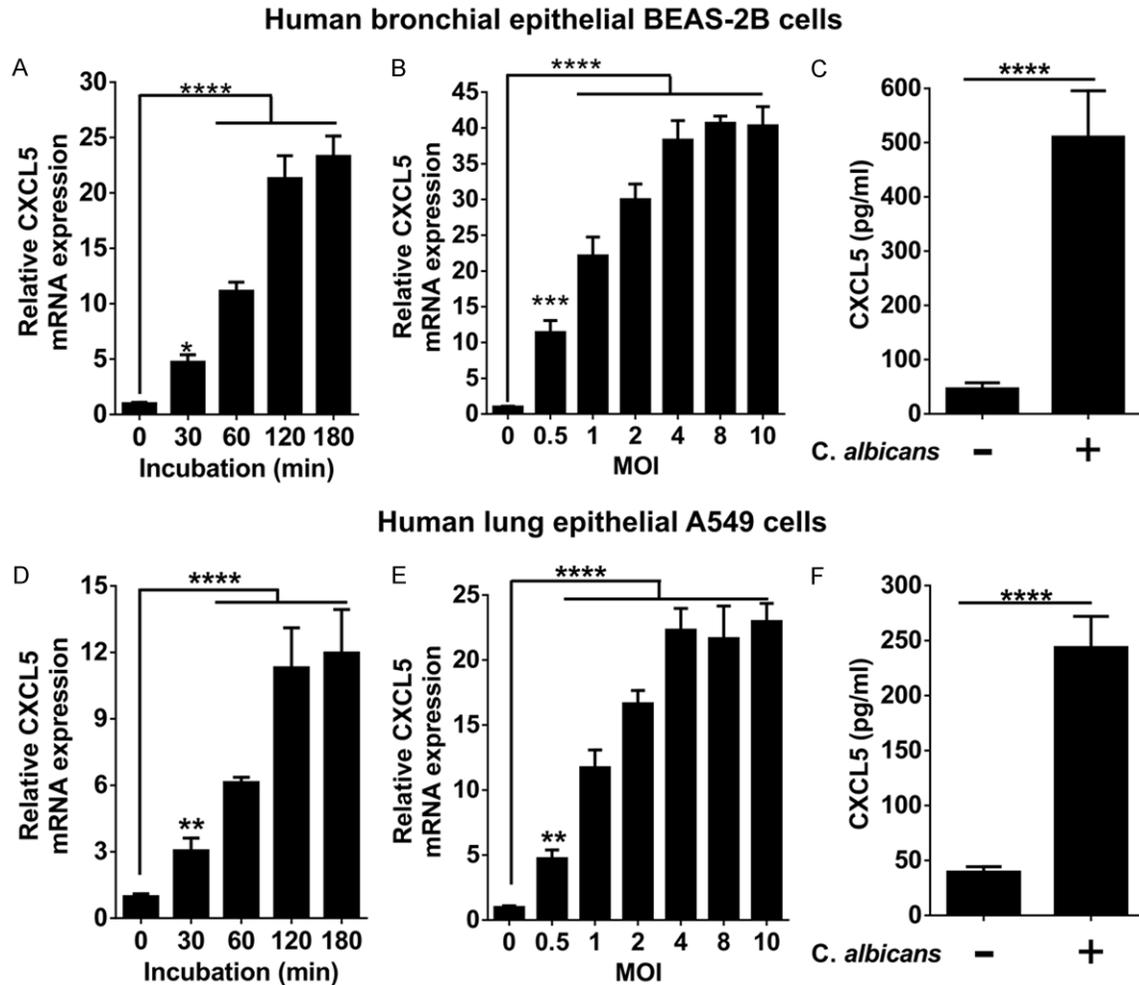
Figure 2. *C. albicans* induces CXCL5 expression in lung epithelial cells. A: Human bronchial epithelial BEAS-2B cells (1×10^5 cells) were incubated with live *C. albicans* (MOI of 1) for different periods, and CXCL5 mRNA expression was measured by qRT-PCR. B: Human bronchial epithelial BEAS-2B cells (1×10^5 cells) were incubated with live *C. albicans* (MOI of 0.5, 1, 2, 4, 8, 10) for 120 min and CXCL5 mRNA expression was measured by qRT-PCR. C: Human bronchial epithelial BEAS-2B cells were incubated with *C. albicans* (MOI of 1) for 120 min and CXCL5 protein levels in cell culture supernatants was measured by ELISA. D: Human lung epithelial A549 cells (1×10^5 cells) were incubated with live *C. albicans* (MOI of 1) for different periods and CXCL5 mRNA expression was measured by qRT-PCR. E: Human lung epithelial A549 cells (1×10^5 cells) were incubated with live *C. albicans* (MOI of 0.5, 1, 2, 4, 8, 10) for 120 min and CXCL5 mRNA expression was measured by qRT-PCR. F: Human lung epithelial A549 cells were incubated with *C. albicans* (MOI of 1) for 120 min and CXCL5 protein levels in cell culture supernatants were measured by ELISA. Data are presented as mean ± SD (n=3). *P<0.05, **P<0.01, ****P<0.0001.

2B cells (**Figure 2B**) and A549 cells (**Figure 2E**) by incubating cells with different doses of live *C. albicans* (MOI, 0.5~10). *C. albicans* also induced upregulation of CXCL5 protein expression in BEAS-2B cells (**Figure 2C**) and A549 cells (**Figure 2F**), as quantified by ELISA. In addition, mRNA and protein expression of CXCL5 in human airway epithelial BEAS-2B cells was higher than those in human lung epithelial A549 cells, in response to *C. albicans* stimulation in same conditions.

C. albicans induces CXCL5 expression depending on activation of NF-κB, p38, and PI3K pathways

Next, we examined whether activation of JNK, PI3K, MEK, p38 MAPK, and/or NF-κB was required for the upregulation of CXCL5 secretion by *C. albicans* in both BEAS-2B cells and A549 cells. Treatment of BEAS-2B cells (**Figure 3A**) and A549 cells (**Figure 3B**) with JNK inhibitor (SP600125) and MEK1/2 inhibitor (U0126) did

Figure 3. *C. albicans* induces CXCL5 expression depends on activation of NF-κB, MAPK p38, and PI3K pathways. A: Human bronchial epithelial BEAS-2B cells (1×10⁵ cells). B: Lung epithelial A549 cells were pretreated with JNK inhibitor (SP600125, 10 μM), MEK1/2 inhibitor (U0126, 20 μM), PI3K inhibitor (LY294002, 20 μM), p38 MAPK inhibitor (SB203580, 20 μM), NF-κB inhibitor (BAY 11-7082, 20 μM) or proteasome inhibitor (MG-132, 20 μM) for 1 h, and then incubated with *C. albicans* (MOI of 1) for 120 min. CXCL5 protein levels in cell culture supernatants were measured by ELISA. Data are presented as mean ± SD (n=3). Inhibitor *vs.* DMSO in *C. albicans*-treated cells, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. C: CXCL5 protein levels in human bronchial epithelial BEAS-2B cells. D: Lung epithelial A549 cells were further reconfirmed by Western blotting.

not inhibit *C. albicans*-mediated upregulation of CXCL5 secretion while PI3K inhibitor (LY294-002), p38 MAPK inhibitor (SB203580), NF-κB inhibitor (BAY 11-7082), and proteasome inhibitor (MG-132) significantly reduced CXCL5 secretion. Furthermore, the same results were further confirmed by Western blotting in both BEAS-2B cells (**Figure 3C**) and A549 cells (**Figure 3D**). Thus, these results suggest that *C. albicans* depends on activation of PI3K, P38 MAPK, and NF-κB pathways to induce CXCL5 secretion by BEAS-2B cells and A549 cells.

Discussion

Neutrophils are the most abundant subpopulation of leukocytes and are essential for maintaining innate immune surveillance. They also represent a major contributor to tissue damage during inflammation caused by invading patho-

gens [14]. Recently, a growing number of studies have been focused on examining the role of CXCL5-driven neutrophil recruitment in infectious diseases. CXCL5 appears to be the dominant effector of neutrophil influx to the lungs. During *mycobacterium tuberculosis* infection, CXCR2 and CXCL5 are strongly upregulated in the lungs [22] and pulmonary epithelial cell-secreted CXCL5 drives destructive neutrophilic inflammation in tuberculosis [13]. Furthermore, CXCL5 exerts a predominant role in mediating neutrophil influx to lungs during inflammation after LPS inhalation and anti-CXCL5 antibodies attenuates LPS-induced neutrophil accumulation in acute lung injury (ALI) [23]. In contrast, high serum levels of CXCL5 also have the potential to increase plasma concentrations of CXCL1/CXCL2, two other receptors for CXCR2, and impair formation of chemokine gradients for neutrophil influx to the lungs by binding to

erythrocyte duffy antigen receptors for chemokines (DARC) [15]. Thus, upregulated secretion of CXCL5 by alveolar type II epithelial (AE II) cells increased lung bacterial burden and mortality in a severe *Escherichia coli* (*E. coli*) pneumonia mouse model [15, 16].

In a mouse model of systemic candidiasis, CXCL5 and CXCR1 were highly induced in *C. albicans*-infected kidneys and CXCR1-dependent neutrophil effector function was shown to be a critical innate protective mechanism of fungal clearance and host survival [10]. However, the mechanisms of how CXCL5 mediates CXCR1-dependent neutrophil effector function remains poorly clarified. In this study, we compared serum levels of CXCL5 in HIV patients with or without pulmonary candidiasis and found that CXCL5 was significantly increased and positively correlated with serum BG levels in HIV patients with pulmonary candidiasis. More importantly, serum CXCL5 levels were markedly decreased after two weeks of Fluconazole treatment, a first-line management option for treatment and prophylaxis of localized and systemic *C. albicans* infection [20, 21]. Thus, *C. albicans* infection induced upregulation of CXCL5 in HIV patients. However, the role of elevated CXCL5 in neutrophils accumulation in patients with pulmonary candidiasis still need to be further clarified in future studies.

Activation of AEII cells via TLR4-dependent p38, STAT3, and c-Jun signaling is important for producing CXCL5 in lungs exposed to LPS [16] or in murine lungs during *E. coli* pneumonia [24]. In addition, non-typeable Haemophilus influenzae (NTHi) upregulates CXCL5 expression by activating NF-κB and p38 MAPK pathways in middle ear epithelial cells [11]. In cellular studies, we have further shown that *C. albicans* infection significantly induced CXCL5 expression in both human lung epithelial cells and bronchial epithelial cells and could be inhibited by presence of PI3K, p38 MAPK and NF-κB inhibitors while the presence of JNK, MEK1/2 showed no significant effects. Thus, activation of PI3K, p38 MAPK, and NF-κB pathways are involved in *C. albicans*-induced CXCL5 expression in lung epithelial cells. TLR2, TLR4, and Dectin-1 are the most important receptors of epithelial cells or immune cells involved in interaction with *C. albicans* [25-27]. Furthermore, these cell surface receptors are also important

upstream molecules of PI3K, p38 MAPK, and NF-κB pathways. Therefore, the detailed molecular mechanisms involved with *C. albicans*-induced CXCL5 expression in lung epithelial cells still need to be clarified in future studies.

In conclusion, for the first time, our study demonstrates that serum CXCL5 is elevated in HIV patients with pulmonary candidiasis. Furthermore, we also confirm that *C. albicans* upregulate CXCL5 expression in both human lung epithelial cells and bronchial epithelial cells by activating PI3K, p38 MAPK, and NF-κB pathways. Hence, the results of our study provide valuable information for a better understanding of the pathogenesis of pulmonary candidiasis.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Wei Chen, Department of Intensive Care Unit, Renmin Hospital of Wuhan University, 238 Jiefang Road, Wuchang District, Wuhan 430060, P. R. China. Tel: +86 15871764649; E-mail: lvxu319@163.com; Dr. Tielong Chen, Department of Infectious Diseases, Zhongnan Hospital of Wuhan University, 169 Donghu Road, Wuchang District, Wuhan 430071, P. R. China. Tel: +86 1355446-6518; E-mail: Tielong.chen@gmail.com

References

[1] Xie LX, Liu SY, Chen YS, Liu K and Xue F. An animal experiment about early pulmonary candidiasis in immunosuppressive rabbits: thin-section CT images dynamically observed and proved by histopathological results. Acta Radiol 2011; 52: 743-749.

[2] Tang XL, Hua Y, Guan Q and Yuan CH. Improved detection of deeply invasive candidiasis with DNA aptamers specific binding to (1→3)-beta-D-glucans from Candida albicans. Eur J Clin Microbiol Infect Dis 2016; 35: 587-595.

[3] Festekjian A and Neely M. Incidence and predictors of invasive candidiasis associated with candidaemia in children. Mycoses 2011; 54: 146-153.

[4] Wahyuningsih R, Freisleben HJ, Sonntag HG and Schnitzler P. Simple and rapid detection of Candida albicans DNA in serum by PCR for di-

agnosis of invasive candidiasis. J Clin Microbiol 2000; 38: 3016-3021.

[5] Wagener J, Weindl G, de Groot PW, de Boer AD, Kaesler S, Thavaraj S, Bader O, Mailander-Sanchez D, Borelli C, Weig M, Biedermann T, Naglik JR, Korting HC and Schaller M. Glycosylation of Candida albicans cell wall proteins is critical for induction of innate immune responses and apoptosis of epithelial cells. PLoS One 2012; 7: e50518.

[6] Rast TJ, Kullas AL, Southern PJ and Davis DA. Human epithelial cells discriminate between commensal and pathogenic interactions with Candida albicans. PLoS One 2016; 11: e0153165.

[7] Herold S, von Wulffen W, Steinmueller M, Pleschka S, Kuziel WA, Mack M, Srivastava M, Seeger W, Maus UA and Lohmeyer J. Alveolar epithelial cells direct monocyte transepithelial migration upon influenza virus infection: impact of chemokines and adhesion molecules. J Immunol 2006; 177: 1817-1824.

[8] Mustapha P, Paris I, Garcia M, Tran CT, Cremniter J, Garnier M, Faure JP, Barthes T, Boneca IG, Morel F, Lecron JC, Burucoa C and Bodet C. Chemokines and antimicrobial peptides have a cag-dependent early response to Helicobacter pylori infection in primary human gastric epithelial cells. Infect Immun 2014; 82: 2881-2889.

[9] Charo IF and Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. N Engl J Med 2006; 354: 610-621.

[10] Swamydas M, Gao JL, Break TJ, Johnson MD, Jaeger M, Rodriguez CA, Lim JK, Green NM, Collar AL, Fischer BG, Lee CC, Perfect JR, Alexander BD, Kullberg BJ, Netea MG, Murphy PM and Lionakis MS. CXCR1-mediated neutrophil degranulation and fungal killing promote Candida clearance and host survival. Sci Transl Med 2016; 8: 322ra310.

[11] Konduru AS, Lee BC and Li JD. Curcumin suppresses NTHi-induced CXCL5 expression via inhibition of positive IKKbeta pathway and upregulation of negative MKP-1 pathway. Sci Rep 2016; 6: 31695.

[12] Lionakis MS, Fischer BG, Lim JK, Swamydas M, Wan W, Richard Lee CC, Cohen JI, Scheinberg P, Gao JL and Murphy PM. Chemokine receptor Ccr1 drives neutrophil-mediated kidney immunopathology and mortality in invasive candidiasis. PLoS Pathog 2012; 8: e1002865.

[13] Nouailles G, Dorhoi A, Koch M, Zerrahn J, Weiner J 3rd, Fae KC, Arrey F, Kuhlmann S, Bandermann S, Loewe D, Mollenkopf HJ, Vogelzang A, Meyer-Schwesinger C, Mittrucker HW, McEwen G and Kaufmann SH. CXCL5-secreting pulmonary epithelial cells drive de-

structive neutrophilic inflammation in tuberculosis. J Clin Invest 2014; 124: 1268-1282.

[14] Mei J, Liu Y, Dai N, Hoffmann C, Hudock KM, Zhang P, Guttentag SH, Kolls JK, Oliver PM, Bushman FD and Worthen GS. Cxcr2 and Cxcl5 regulate the IL-17/G-CSF axis and neutrophil homeostasis in mice. J Clin Invest 2012; 122: 974-986.

[15] Mei J, Liu Y, Dai N, Favara M, Greene T, Jeyaseelan S, Poncz M, Lee JS and Worthen GS. CXCL5 regulates chemokine scavenging and pulmonary host defense to bacterial infection. Immunity 2010; 33: 106-117.

[16] Jeyaseelan S, Manzer R, Young SK, Yamamoto M, Akira S, Mason RJ and Worthen GS. Induction of CXCL5 during inflammation in the rodent lung involves activation of alveolar epithelium. Am J Respir Cell Mol Biol 2005; 32: 531-539.

[17] Luo F, Chen T, Liu J, Shen X, Zhao Y, Yang R and Zhang X. Ficolin-2 binds to HIV-1 gp120 and blocks viral infection. Virol Sin 2016; 31: 406-414.

[18] Zhao Y, Ren Y, Zhang X, Zhao P, Tao W, Zhong J, Li Q and Zhang XL. Ficolin-2 inhibits hepatitis C virus infection, whereas apolipoprotein E3 mediates viral immune escape. J Immunol 2014; 193: 783-796.

[19] Zhou SL, Dai Z, Zhou ZJ, Wang XY, Yang GH, Wang Z, Huang XW, Fan J and Zhou J. Overexpression of CXCL5 mediates neutrophil infiltration and indicates poor prognosis for hepatocellular carcinoma. Hepatology 2012; 56: 2242-2254.

[20] Martin MV. The use of fluconazole and itraconazole in the treatment of Candida albicans infections: a review. J Antimicrob Chemother 1999; 44: 429-437.

[21] Lyon GM, Karatela S, Sunay S and Adiri Y. Antifungal susceptibility testing of Candida isolates from the Candida surveillance study. J Clin Microbiol 2010; 48: 1270-1275.

[22] Slight SR and Khader SA. Chemokines shape the immune responses to tuberculosis. Cytokine Growth Factor Rev 2013; 24: 105-113.

[23] Jeyaseelan S, Chu HW, Young SK and Worthen GS. Transcriptional profiling of lipopolysaccharide-induced acute lung injury. Infect Immun 2004; 72: 7247-7256.

[24] Traber KE, Hilliard KL, Allen E, Wasserman GA, Yamamoto K, Jones MR, Mizgerd JP and Quinton LJ. Induction of STAT3-dependent CXCL5 expression and neutrophil recruitment by Oncostatin-M during pneumonia. Am J Respir Cell Mol Biol 2015; 53: 479-488.

[25] Xu R, Sun HF, Williams DW, Jones AV, Al-Hussaini A, Song B and Wei XQ. IL-34 suppresses Candida albicans induced TNFalpha production in M1 macrophages by downregulating

expression of Dectin-1 and TLR2. J Immunol Res 2015; 2015: 328146.

[26] Yanez A, Megias J, O'Connor JE, Gozalbo D and Gil ML. Candida albicans induces selective development of macrophages and monocyte derived dendritic cells by a TLR2 dependent signalling. PLoS One 2011; 6: e24761.

[27] Pinke KH, Lima HG, Cunha FQ and Lara VS. Mast cells phagocyte Candida albicans and produce nitric oxide by mechanisms involving TLR2 and Dectin-1. Immunobiology 2016; 221: 220-227.