Microbial Pathogenesis 91 (2016) 115–122

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Candida glabrata induced infection of rat tracheal epithelial cells is mediated by TLR-2 induced activation of NF-κB

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ARTICLE INFO

Article history:
Received 11 May 2015
Received in revised form
12 October 2015
Accepted 13 October 2015
Available online 23 October 2015

Keywords:
Invasive pulmonary fungal infection
Candida glabrata
TLR
PAMP

ABSTRACT

An increasing number of reports identified *Candida glabrata* (*C. glabrata*) as the important causative agent of invasive pulmonary fungal infection. However, little is known about immune responses to *C. glabrata* in rat tracheal epithelial cell (RTEC). Here, the effect of *C. glabrata* on RTEC and the role of TLR-2 and NF-κB in the immune response were investigated by treatment with TLR-2 siRNA and NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC), respectively. Our results showed that the knockdown of TLR-2 and pretreatment of PDTC led to inhibition of cell proliferation by *C. glabrata*, further enhanced cells in G0/G1 phases, and promoted *C. glabrata* -induced apoptosis. *C. glabrata* infection induced the expression or secretion of TLR-2, NF-κB, TNF-α, and IL-6, and its effect was inhibited by knockdown of TLR-2. Pretreatment with PDTC inhibited the *C. glabrata* -induced expression of TLR2, and also inhibited the expression of p65 subunit of NF-κB in the first 4 h. Although the expression of p65 subunit at 6 h was elevated compared to baseline, the *C. glabrata* -induced expression of TNF-α and IL-6 remained attenuated by PDTC pretreatment. Therefore, *C. glabrata* recognized the TLR-2 in rat tracheal epithelial cell (RTEC), and then activated the transcription factor NF-κB and further promoted the secretion of TNF-α and IL-6 to contribute to the immune response and inflammation.

1. Introduction

Candidemia is the fourth most common cause of hospital acquired infection. Despite the availability of effective antifungal therapy, mortality due to invasive fungal infection (IFI) remains very high, especially in immune compromised patients such as critically ill patients, AIDS patients in Intensive Care Units (ICU) or patients undergoing cancer therapy [1–4].Invasive pulmonary fungal infection(IPFI) is the most common form of fungal infection [4]. As a result of the wide application of azoles, echinocandins, allylamines and polyenes as antifungal prophylaxis, resistance is easily developed in clinical Candida Isolates. In fact, Candida species are one of the most commonly detected organisms in the IPFI [5]. Although *Candida albicans* (*C. albicans*) remains the most common cause of IPFI at many institutions, non-albicans species such as *Candida glabrata* (*C. glabrata*), Candida tropicalis, Candida parapsilosis and Candida kruseiare increasingly associated with pulmonary candidiasis [4,6–9].Among the non-albicans species, *Candida glabrata (C.glabrata)* was identified as the most important causative agent in an increasing number of reports [10–13]. However, little is known about immune responses to *C. glabrata* in tracheal epithelial cell (TEC).

The innate immune response is the host's first line of defense against infectious disease by recognizing the invading pathogens early and triggering an appropriate pro-inflammatory response [14]. The innate immune system utilizes a limited number of germline-encoded pattern recognition receptors (PRRs) to recognize evolutionary conserved structures on pathogens, named pathogen-associated molecular patterns (PAMPs) [14]. The most extensively studies class of PRRs are the Toll-like receptors (TLRs), a family of 10 proteins in human [15,16]. TLRs play important roles in dorsoventral patterning and antifungal responses. PAMP recognition by TLRs leads to activation of transcription factors such as

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http://dx.doi.org/10.1016/j.micpath.2015.10.009
0882-4010/

nuclear factor-kappa B (NF-κB), which translocates to the nucleus and initiates transcription of cytokine genes. Then, the release of cytokines promotes the accumulation of neutrophils, macrophages, lymphocytes, and monocytes at the site of infection to destroy pathogenic fungi. The TLR family members are expressed on various immune and non-immune cells such as B-cells, NK (naturalkiller) cells, dendritic cells (DCs), macrophages, fibroblast cells, epithelial cells and endothelial cells [16]. However, TLRs are differentially localized within the cells. TLR-2 recognizes a structurally diverse range of PAMPs and complete pathogens. There is limited understanding of the role of TLR-2 in *C. glabrata*-infected TECs.

In the present study we will focus on the mechanisms involved in *C. glabrata* infected rat tracheal epithelial cells (RTEC).We found that the expressions of TLR-2, NF-κB, TNF-α, and IL-6 in *C. glabrata*-infected rat tracheal epithelial cell were increased with increased duration of infection. Then, the roles of TLR-2 and NF-κB in immune response were investigated by treating the cells with TLR-2 siRNA and NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC).

2. Methods and materials

2.1. Strains and culture

The study was carried out using *Candida glabrata* (*C. glabrata*, No.9527), which was obtained from Shanghai Enzyme-linked Biotechnology Co., Ltd., China. The fungal specific internal transcribed spacer (ITS) region was used for confirmation of *C. glabrata* strain. The strain was cultured on Yeast Extract-Peptone-Dextrose (YPD) agar and incubated aerobically at 37 °C.The optimum concentration of *C. glabrata* (10^7 CFU/ml) was used to infect the cells by a preliminary test in Fig. S1.

2.2. Cell culture

Primary rat tracheal epithelial cells (RTECs) were cultured as previously described [17,18]. Briefly, RTECs were obtained from male Fisher 344 rats by overnight digestion with pronase and plated at 3×10^4 cells/cm^2 on collagen-gel-coated porous membranes. The cultures contained 2.5 ml of media in the lower (basal) compartment and 0.5 ml of media in the upper (apical) compartment for the first 7–9 d of culture. Then, cells in the basal compartment were collected and subcultured for the experiments. After infection with *C. glabrata* for 2 h, 4 h, or 6 h, proliferation, cell cycle and apoptosis assays were performed, and the expression of TLR-2, NF-κB, TNF-α, and IL-6 were determined.

2.3. Cell transfection

The role of TLR-2 in *C. glabrata* -infected TECs was examined using siRNA-mediated knockdown of TLR-2. For transfection of TLR-2 siRNA (50 ng/ml, 100 ng/ml, and 200 ng/ml) ornon-targeting negative control (Santa Cruz, USA), DharmaFECT reagent (Life Technologies, USA) was used according to the manufacturer's instructions. After 48 h, the culture medium was replaced with fresh supplemented medium, and the cells were cultured in the presence of *C. glabrata* (10^7 CFU/ml) for 2 h, 4 h or 6 h. Then, proliferation, cell cycle and apoptosis assays were performed, and the expression of TLR-2, NF-κB, TNF-α, and IL-6 were determined.

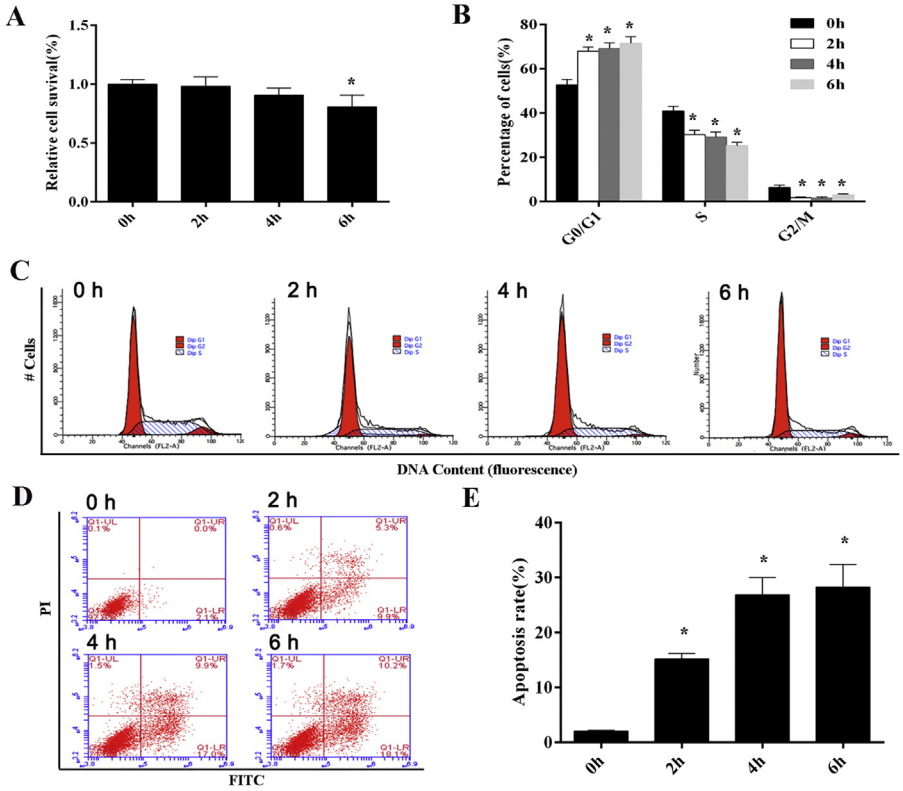

Fig. 1. Cell proliferation, cell cycle and apoptosis analysis of RTECs after *C. glabrata* infection. Following *C. glabrata* infection, (**A**) the proliferation of RTECs was measured. Also, (**B**) the percentage of cells in the G0/G1, S and G2/M phases, and (**C**) cell cycle distribution was measured by flow cytometry using the cell cycle staining kit (MultiSciences, China); $^*P < 0.05$ vs. 0 h. (**D**) Cell apoptosis was determined using the Annexin V-FITC/PI flow cytometry, and (**E**) the proportion of apoptotic cells was measured.