Original Article

Adherence and Cytotoxicity of Candida spp. to HaCaT and A549 cells

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ABSTRACT

Candida species are opportunistic fungal pathogens that cause superficial or invasive infections. Recently, the incidence of infection by non-Candida albicans species, especially Candida glabrata, has increased. In this study, we analyzed the adhesion and cytotoxicity of various Candida spp. that are part of the normal human microbiota. C. albicans adheres well to cell culture plates and to cultured cells. C. glabrata selectively adheres to epithelial cells rather than to cell culture plates. Candida parapsilosis insufficiently adheres to confluent monolayers of human lung epithelial A549 and keratinocyte HaCaT cells. We then analyzed the cytotoxicity of C. albicans and C. glabrata, which adhered well to epithelial cells. C. glabrata has been found to cause more damage to A549 cells than to HaCaT cells, suggesting that resident Candida spp. have distinct cytotoxic effects in different tissues. It is important to clarify the properties of Candida spp. as there is evidence that normal microbiota can cause infections. Our data suggest that it is necessary to use appropriate cell lines for characterizing the adherence and cytotoxicity of Candida spp.

Key words: adhesion, C. albicans, C. glabrata, cytotoxicity

Introduction

Candida species are major fungal pathogens that cause skin, mucosal, and deep-seated infections. Increasing rates of infection by non-Candida albicans species are being reported, especially by Candida glabrata, although C. albicans is still the most commonly isolated species from blood samples¹. Previous studies in Japan and Italy demonstrated that C. albicans was the most prevalent species, followed by Candida parapsilosis, C. glabrata, and Candida tropicalis²⁻⁴. Moreover, it has been reported that the use of the antifungal agents fluconazole and caspofungin increases the proportions of C. glabrata and Candida kefyr⁵. Several virulence factors that contribute to the pathogenic potential of Candida spp. have been identified. Of these factors, the ability to adhere to epithelial cells is key as it contributes to the first step in colonization or infection. Candida spp. have been isolated from several anatomically distinct sites (oral cavity, skin, etc.) of healthy adults⁶ and from foot ulcers and skin and nail samples from patients with diabetes mellitus⁷. Furthermore, Candida spp. can cause candidal aspiration pneumonia in the oral cavity⁸⁻¹⁰. Conversely, the adhesion of these pathogens to abiotic surfaces is also problematic because it can lead to the formation of biofilms on medical devices, such as catheters. These cutaneous infections, candidal pneumonia, and catheter-related infections might be caused by resident Candida spp., thus it is important to analyze the features and pathogenicity of Candida spp. in several host tissue cell types. There are not many reports of adhesion assays using multiple strains of each species. In this study, we analyzed the adhesion of Candida spp. to human epithelial cells as well as their cytotoxicity using human keratinocyte HaCaT cells as epidermal epithelial cells that can come in contact with normal microbiota and using human lung epithelial cells (A549).

Materials and methods

Strains and cell lines

C. albicans (CBS562, SC5314, J1-97, J2-36, MK0201, and
MK0601), C. parapsilosis (J2-118, J2-127, J2-132, CBS1954, and MK0502), and C. glabrata (4021, 4019, 4018, and CBS138) were used. We collected MK0201, MK0601, and MK0502 from healthy adults. Swab samples were taken from the oral cavity, and species were identified based on colonial color on a CHROMagar Candida Plate (Kanto Chemical Co., Inc., Tokyo, Japan) and Vitalmedia Color Candida Agar (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan) and by sequence analysis of the yeasts’ internal transcribed spacer regions. All samples were taken after obtaining informed consent. The study protocol was approved by the Ethical Committee of our university.

As549 human lung epithelial cells and HaCaT human keratinocyte cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C with an atmosphere supplemented with 5% CO2. All experiments were carried out using 24-well tissue culture plates. Cultures were maintained at 37°C with 5% CO2, cultured media were assayed for LDH activity induced by C. glabrata against A549 was higher than that against HaCaT cells (p < 0.05).

**Results**

**Adhesion of Candida spp. to cell culture plates and to human epithelial cells**

C. albicans and C. parapsilosis adhered to the surface of a cell culture plate and formed hyphae, although the degree of adherence was different for each strain (Fig. 1a). Absorbance of the extracted CV was measured at 550 nm. The absorbance of C. albicans and C. parapsilosis, which produce hyphae, was higher than that of C. glabrata because the staining reflects the cellular volume (Fig. 1b).

In the one-day adherence assay on epithelial cells, C. albicans and C. glabrata attached to the surface of A549 and HaCaT cells. A representative photograph is shown in Fig. 2. C. albicans grew as a hyphal colony. Four C. parapsilosis strains hardly adhered to epithelial cells, although these strains rarely form a hyphal colony on the epithelial cells. C. albicans and C. glabrata strains also adhered to A549 cells (data not shown).

Next, the adherence of C. albicans was compared to that of C. glabrata in the one-hour adherence assay. C. albicans cells formed germ tubes and were well attached to the cell culture plates after 1 h of incubation, whereas C. glabrata showed weak adherence (Figs. 3a and 3b). The one-hour adherence assay on epithelial cells revealed that C. glabrata attached to A549 and HaCaT cells more than C. albicans did. C. albicans did not demonstrate selectivity for mammalian cells, while C. glabrata selectively adhered on or around mammalian epithelial cells (Figs. 3c and 3d).

**Cytotoxicity**

C. albicans and C. glabrata cells cultured on HaCaT and A549 cells caused cytotoxicity, whereas yeast cells that did not come in contact with these cells hardly induced cytotoxicity (Fig. 4). C. glabrata showed cytotoxicity comparable to or higher than that of C. albicans. The LDH activity induced by C. glabrata against A549 was higher than that against HaCaT cells (p < 0.05).
Discussion

Many studies have been carried out on the adherence of *C. albicans* to plastic or silicone surfaces and to mammalian cells. Some previous reports have demonstrated that the adhesion of *C. albicans* to plastic or mammalian cells is greater than that of non-*C. albicans* species. In the present study, we showed that *C. glabrata* cells adhered more selectively to mammalian cells than to cell culture plates, and *C. parapsilosis* insufficiently adhered to confluent epithelial cells. Some reports have indicated that the adherence activity of *C. parapsilosis* is lower than that of *C. albicans*, but in some cases the opposite has been documented. In some reports, the sample size was small, and one or two strains were used for adhesion assay to compare the adhesion of *Candida* spp. Furthermore, the yeast strain or the kind of epithelial cells might affect the adherence ability. We used at least four strains of each *Candida* spp. for adhesion assay in our study to determine the general tendency for each species.

Different adhesins might affect the selectivity of yeast cell attachment to materials or cellular surfaces, and synergistic interactions of multiple adhesins might determine this selectivity. Various adhesins of *Candida* spp. have been reported, such as agglutinin-like sequence (Als) family protein, epithelial adhesin (Epa), and hyphal wall protein 1 (Hwp1). These proteins might interact with epithelial cell factors. Epa proteins of *C. glabrata* have been reported to...
Fig. 2. One-day adhesion assays on epithelial cell monolayers. Yeast cells were inoculated into epithelial cells with DMEM supplemented with 1% FBS. Adherent yeast cells were imaged by microscopy at 40 × (a) and 400 × (b) magnification.

Fig. 3. One-hour adhesion assays on cell culture plates (a, b) and epithelial cells (c, d). (a) Adherent yeast cells were imaged by microscopy at 400 × magnification. (b) CV staining of adherent cells. Yeast cells were inoculated into A549 (c) and HaCaT (d) cells with DMEM supplemented with 1% FBS. Adherent yeast cells were imaged by microscopy at 400 × magnification. The arrowheads point to the adhered yeast cells.
interact with epithelial cells, and Epa1 as well as Epa7 mediate adherence to epithelial cells better than Epa6 does. Thus, each protein’s function in adherence is different among the adhesin molecules. On the other hand, transcript levels of Epa6 and Epa7 are elevated during biofilm formation.

We analyzed cytotoxicity using inter-cell chambers to create two conditions: one in which yeast cells come in contact with mammalian epithelial cells and the other in which the yeast did not come in contact with the epithelial cells directly. Soluble factors may affect cytotoxicity because Candida spp. produce proteolytic enzymes such as secreted aspartyl proteases. However, in our study, cytotoxicity was detected only after the direct interaction of host cells with yeast cells. Moreover, A549 cells were more damaged by the addition of C. glabrata than HaCaT cells were (p < 0.05), suggesting that the alveolar epithelium may be more susceptible to infection by C. glabrata. It has been reported that C. glabrata induces cytokines in human oral epithelia in a strain-specific manner. Slight strain differences in adhesion to culture plate surfaces were evident in our assays, and strain differences were also detected in cytotoxicity, especially with C. albicans. However, adhesive strength and cytotoxicity did not have a clear correlation.

Strain-dependent cell damaging ability may be affected by pathogenic factors. Factors other than adhesive ability may contribute to cell damage, but we could not determine whether the strain differences were related to other pathogenic factors.

We did not analyze the cytotoxicity of C. parapsilosis against epithelial cells. However, the cell damage by C. parapsilosis was surmised to be weak because yeast cells that did not come in contact with epithelial cells insufficiently induced cytotoxicity in both C. albicans and C. glabrata. These Candida spp. are part of the normal human microbiota. C. glabrata was isolated from the oral cavity, and there are reports that C. glabrata is isolated more frequently from the elderly than from the young. Since aspiration pneumonia attributable to C. glabrata and C. albicans has been reported, the characterization of resident Candida spp., including those in the oral cavity, is important. Our results suggest that Candida spp. that have a lower biofilm-forming ability may adhere strongly and induce cytotoxicity to clinically aseptic host tissues, and that it is necessary to use appropriate cell lines to characterize their properties.

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Conflicts of interest

None declared.

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