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EC MICROBIOLOGY
Research Article

Expression and Releasing of Cell Cycle Proteins by *Candida albicans* into Surrounding Tissue: New Perspectives of the Relationship between Microbes and Host

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Received: December 12, 2018; **Published:** February 14, 2019

Abstract

The present study intends to introduce new philosophical perspectives of how *Candida albicans* (*C. albicans*) can interact with host cells. We depended on introducing a new approach in which *C. albicans* expresses and releases important proteins such as estrogen receptor. This approach was examined using immunohistochemical techniques. Rats were peritoneally injected with *C. albicans* taken from nutrient broth medium. Two weeks later, rats were killed using anesthetic techniques. The results revealed the expression and releasing of ER in *C. albicans* in either of its cellular or hyphal forms. We noticed the existence of ER as exudate from the areas of infection towards host cells. Taken together, up to the best knowledge of the researcher, this is the first study to report the interaction between *C. albicans* and host cells through the expression and releasing of ER which may hinder the stability of host cells and induction of disease development and progression. The data of our study may need to be further confirmed using more sophisticated techniques.

Keywords: *Candida albicans; Estrogen Receptor; Expression; Releasing; Inflammation*

Introduction

C. albicans exists in oral cavity of humans as a commensal flora in about 50% of persons [1,2]. It exists mainly in spherical form and can exhibit filamentous hyphal form according to environmental conditions [3,4]. The ability to switch in its forms determines its pathogenicity since it is associated with the expression of several fungal pathogen-associated molecular patterns (PAMPs) [2,4,5]. PAMPs can be recognized by host cells, which, in turn, can induce signaling pathways as well as cellular responses such as production and releasing of cytokines [6].

Candida species, known as opportunistic pathogens, can cause a variety of disease including simple mucocutaneous infections and more complicated forms of fungemia leading to death. Because *C. albicans* is the most fungal associated with diseases, it is the most studied species. Furthermore, *C. albicans* works to lower the immunity response of host and complicates the pathogenicity control [7].

Candida spp. is considered part of the normal microbiota [8]. Several studies have pointed out to the existence of certain factors that can interact with host and to change the status of being commensal to pathogenic form of yeast [9,10]. *C. albicans* has a very complicated set up of virulence factors to survive, growth and pathogenicity, such as Secreted Aspartyl Proteinases (SAP); surface adhesins, phospholipases; in addition to the ability of forming hyphae and biofilms [11].

In this study, we are introducing a unique approach of interaction between *C. albicans* and host cells.

Estrogen and *C. albicans*

According to environmental conditions such as the presence of estrogen, *C. albicans* can transit its cellular form to hyphal form (the pathogenic form) [12], due to having a biochemical system involving the existence of a set of proteins that can interact with steroids such as estrogen binding protein (Ebp1) [4,12-14]. We have recently demonstrated that this Ebp1 is an estrogen receptor [4].

Human studies showed that the estrogens such as 17β-estradiol have the ability to control both proliferation and differentiation. Researchers found that 17β-estradiol induced morphological changes of *C. albicans* from the yeast-to-hypha via EBP1 in *C. albicans* [15]. According to this context, the presence of high levels of estrogen is thought to be risk factor for disease development and progression [16,17]. Other studies confirmed this trend through reporting the existence of the association between increased estrogen levels in pregnancy period and increased vaginal colonization with *C. albicans* [4,12,18,19]. In addition to the above context, several studies have reported that the use of exogenous estrogens such as oral contraceptives increased colonization of *C. albicans* [4,12,18,19].

Study Objective

The main objective of this study is to follow up the released biomarkers (ER) from the area in which *C. albicans* exists towards the host cell.

Methodology

We followed the procedure that was reported in our previous study [4]. We cultured *C. albicans* (ATCC) in Sabouraud Dextrose Broth (SAB) for 24 hrs at 37°C. A broth medium (SAB) tube containing *C. albicans* was centrifuged to concentrate the amount of *C. albicans* for 5 minutes (3000 RPM). We took a sample of the sediment diluted in sterilized normal saline 1:10 ratio and rats were intraperitoneally injected. After two weeks, rats were killed using anesthetic conditions. Tissues were taken and fixed in 10% neutral formalin for 24 hrs. Processing of the tissues was carried out using tissu**e** processor overnight. Tissues were then embedded in paraffin and sections (3μ) mounted on charged slides were obtained using a rotatory microtome (Leica 2135).

Immunohistochemistry

Sections were de-paraffinized using oven at 65°C for an hour. The slides then were passed into solutions through xylene, 100% ethanol, 90% ethanol, 80% ethanol, 70% ethanol (5 minutes each), and distilled water. Sections were immersed in 1% hydrogen peroxide in absolute methanol for 20 minutes to lower or inhibit endogenous peroxidase activity. Slides then were washed with phosphate buffer saline (pH = 7.2 - 7.4) for 5 minutes and then incubated with 1% bovine serum albumin for 30 minutes to minimize or prevent non-specific bindings. During that time, the primary antibody and other immunohistochemistry reagents were prepared and brought to room temperature. The monoclonal antibody (estrogen receptor), (Dako, code M7047, clone 1d5) was prepared (1:100) and incubated with slides for an hour in humid chamber. After that, slides were washed with phosphate buffer saline (pH = 7.2 - 7.4) for 5 minutes, then incubated with secondary biotinylated antibodies for 20 minutes, then washed with phosphate buffer saline (pH = 7.2 - 7.4) for 5 minutes, then incubated with streptavidin conjugated with horseradish peroxidase enzyme for 20 minutes, then washed with phosphate buffer saline (pH = 7.2 - 7.4) for 5 minutes. Finally, immune-histochemical reactions were visualized through incubation with DAB (diaminobenzidine) till the development of brown reaction, and then sections were washed with tab water to stop the reaction. Section then were stained with hematoxylin for 30 seconds as a counterstain, then sections were washed with water, dehydrated and mounted with mounting medium. These protocols were described in other studies [20,21].

Results

The results of the present study can be described as follows:

1. The infected areas of tissues confirmed the existence of *C. albicans* in both cellular and hyphal forms. This was clear in different tissues including liver and ovaries as stained by (H&E).
2. The infected areas exhibited inflammatory reactions as infiltration of white blood cells.
3. ER was localized in *C. albicans* and released into adjacent areas.
4. It was observed that ER was expressed in host cells.

Discussion

The present study introduced a unique approach explaining the interactions between *C. albicans* and host cells far away from what has been thought. We think that this is the first report describing such mode of interaction.

The relationship between the existence of estrogen and candidiasis has been reported [4,12,16,17]. We think that through the expression and releasing of proteins such as ER, *C. albicans* interferes and impacts cell cycle. The host cell may not tolerate more estrogens and become more likely to develop disease and progression.

Conclusion

Up to the best knowledge of the researcher, this is the first study to report the interaction between *C. albicans* and host cells through the expression and releasing of ER which may hinder the stability of host cells and induction of disease development and progression**.**

Recommendations

The data of our study may need to be further confirmed using more sophisticated techniques.

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Volume 15 Issue 3 March 2019