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Phage vaccines displaying YGKDVKDLFDYAQE epitope induce protection against systemic candidiasis in mouse model

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ABSTRACT

Candida albicans is a common commensal and opportunistic fungal pathogen in human, which poses threat to human health, especially in immunocompromised patients. Unfortunately, few effective prophylactic and therapeutic strategies were applied to clinic practice. Recently, the peptide YGKDVKDLFDYAQE from Fructose-bisphosphate aldolase 1 (Fba1), as a vaccine, was reported to induce protection effects against systemic candidiasis. Here, we displayed this epitope peptide on the coat proteins (pIII or pVIII) of filamentous phage, and investigated their protective effects against *C. albicans* infections. Mice were immunized with recombinant phages (designated as phage-3F and phage-8F) or protein (rFba1), then challenged with *C. albicans* yeast cells via lateral tail vein. Results demonstrated that the recombinant phages as well as rFba1 apparently induced humoral and cellular immune responses, reduced fungal burden and relieved kidney damage in infected mice and significantly improved their survival rates. Briefly, all these findings indicated that the recombinant phages displaying the epitope YGKDVKDLFDYAQE have the potential to be developed into a new vaccine against *C. albicans* infections.

1. Introduction

Candida species are well-known pathogens which can lead to hospital-acquired bloodstream infections in the world [1–4]. Among them, *Candida albicans* (*C. albicans*) is an opportunistic fungal pathogen which exists in normal organisms, but causes severe bloodstream infections (called candidemia) in immunocompromised hosts, such as cancer and AIDS patients; people suffered from major surgeries; or people administrated with immunosuppressive drugs [5,6]. The mortality of systemic candidiasis remains as high as 81% after current antifungal therapy [7–9]. Therefore, there is an urgent need to develop more effectively prophylactic and therapeutic reagents.

Currently, vaccine strategies to increase host resistance are drawing widespread attentions [10–13]. Experimental and clinical evidences demonstrated that vaccination in susceptible population is a feasible strategy to prevent invasive Candida infection [14]. Peptide vaccines consist of one or more epitopes of some specific antigens, those epitopes are then conjugated into a carrier to acquire desired immunogenicity [15]. Peptide vaccines are the most extensively studied types of fungal vaccines and have the potential to be an ideal vaccine [15–17].

Fructose-bisphosphate aldolase (Fba1) is a critical enzyme involved in the glycolytic process [18,19]. Previous studies showed that *Fba1* was highly expressed when hyphal formed and invaded into internal organs, and depleting Fba1 attenuated the virulence of *C. albicans* in the mouse with systemic candidiasis [20,21]. Other studies indicated that peptide YGKDVKDLFDYAQE derived from Fba1 was a more effective epitope for the vaccine against *C. albicans* infections than those derived from other cell proteins [15–17].

Phage display technology was used and a filamentous phage was employed as a vector to display the peptide epitope in this study. The filamentous phage consists of five structural proteins, including one major coat protein (pVIII) making up the longitudinal backbone of the phage and four minor coat proteins constituting the head and tail of the phage [22,23]. There are two practical display systems: for the pIII display system, the foreign peptide is fused to the N-terminal of all pIII copies; and for the pVIII display system, the foreign peptide is fused to the N-terminal for about 150 copies of pVIII because of the steric hindrance [24–26]. Both display systems were employed in this study, the epitope peptide from Fba1 aforementioned was displayed on pIII and pVIII to yield two recombinant phages, which were designated as phage-3F and phage-8F, respectively. Series assays were used to estimate their protective functions including immune response, kidney fungal

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colonization, histopathological examination and the survival rate with recombinant Fba1 (rFba1) and wild type phage as positive and negative controls, respectively. Results showed that phages displaying peptide demonstrated an apparent protective role in infected mice, making it a promising candidate vaccine in systemic candidiasis. In addition, the results further supported that phage display technology is a valid method for vaccines design with the advantages of safety, low cost and convenience for mass production [27–29].

2. Materials and methods

2.1. Ethics statement

All animal experiments were performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of China Association for Laboratory Animal Science and were approved by the Ethics Committee of Northeast Normal University.

2.2. Candida strains and culture conditions

To harvest stationary-phase yeast cells, *C. albicans* (ATCC 10231) was grown on YPD broth (1% peptone, 1% yeast extract, 2% glucose) at 37 °C overnight. One portion of yeast cells were washed and suspended in PBS with an appropriate cell concentration (8×10^5/ml for sublethal, 5×10^6/ml for lethal dose) to infect mice. To obtain hyphal cells, another portion of yeast cells were inoculated in liquid RPMI 1640 medium supplemented with 10% fetal bovine serum and kept at 37 °C for 90 min. The hyphal cells were further used for immunofluorescence staining.

2.3. Construction of plasmids for the production of rFba1

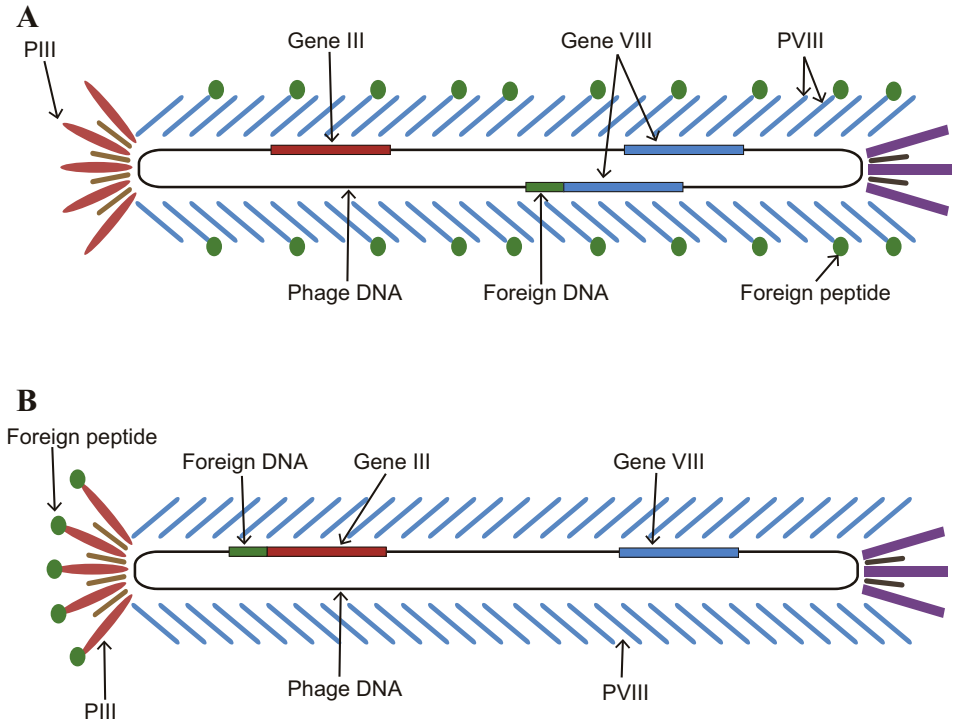
C. albicans RNA was obtained and reversely transcribed into first strand cDNA. *Fba1* gene was cloned and inserted into pET28a$^+$ vector for sequencing confirmation. The recombinant plasmid was subsequently transformed into *E. coli* BL21(DE3). Histidine-tagged rFba1 was extracted and then purified using nickel-based affinity chromatography according to manufacturer's instructions (GE, USA). After dialysis in PBS, the rFba1 protein was quantified by spectrophotometry and subjected to SDS-PAGE and sliver staining to detect its purity.

2.4. Construction of plasmids for the production of phage-3F and phage-8F

As an epitope, the 14-mer peptide (YGKDVKDLFDYAQE) derived from Fba1 was displayed on the coat proteins of filamentous phages. A fUSE55 vector was used to display the epitope on all copies of minor coat protein pIII (called phage-3F), while the f88-4 vector displays the epitope on a fraction copies of the pVIII protein (called phage-8F) (Scheme 1). Vector construction and preparation of recombinant phage were described previously [30,31]. Briefly, the complementary oligonucleotides for the 14-mer peptide were inserted into the phage vector fUSE55 and f88-4 respectively, and then the recombinant vectors were transformed into *E. coli* K91BlueKan, which were further cultured in LB liquid medium (1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was required for phage-8F but not for phage-3F) supplemented with tetracycline. Then, the cultured supernatants containing the phage particles were collected and purified in polyethylene glycol-6000 solution (5% PEG-6000, 0.5 M NaCl) after two consecutive precipitations. Subsequently, the pellets were resuspended in 2 ml PBS, and the phage concentration was measured by spectrophotometry. Finally, the epitope sequence displayed on phage was confirmed by SDS-PAGE followed by silver staining and Western blot analysis.

2.5. The production of polyclonal antibodies

For the first immunization, rFba1 was emulsified with an equal volume of Freund's complete adjuvant, whereas Freund's



Scheme 1. Schematic diagram of the structures of phage-8F and phage-3F. (A) Phage-8F, the f88-4 vector displays the epitope on fraction copies of the major coat protein pVIII. (B) Phage-3F, the fUSE55 vector displays the epitope on all copies of minor coat protein pIII.

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