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The Studies on the Expression and Function of NADPH Oxidase Genes from *Candida* Species during Infection of Human Hepatocytes and under Oxidative Stress

Chapter 1 Introduction

The pathogenic fungi *Candida albicans* and *C. glabrata* rank as the first and the second leading cause of candidiasis. It is reported that they cause hepatic cell apoptosis by producing reactive oxygen species (ROS). The NADPH oxidase genes (*CgNOX1* and *CaFRE8*), which generate the ROS in these fungi, play a critical role during this process. Moreover, the role of *NOX* genes from *C. glabrata* under oxidative stress remains unknown.

The study aimed to elucidate the role of a putative *NOX* gene in *C. glabrata* (named *CgNOX1*) during the co-incubation with human hepatocytes and under oxidative stress and investigate promoters of *CgNOX1* and *CaFRE8* in the transcriptional regulation under these specific conditions. Using the RT-qPCR technique, the transcription expression of relative genes was analyzed. Moreover, I established several GFP-tagged *Candida* recombinants derived by *CgNOX1* promoter and *CaFRE8* promoter. This finding has the potential to serve as a basis for the treatment or prevention of liver injury caused by *C. albicans* through the regulation of the *CaFRE8* promoter region.

Chapter 2 The role of the *CgNOX1* gene in oxidative stress response and in a hepatic infection model.

In this chapter, I investigated the role of *CgNOX1* during the co-cultivation of hepatocytes (HC cells) and oxidative stress. It is observed that there is a significant increase in *CgNOX1* transcript level after 24 h co-incubation with HC cells or treatment with hydrogen peroxide.

Moreover, ROS level detection also revealed that the wild-type and *CgNOX1* reintegrated strain exhibited higher levels of ROS under H_2O_2 stress. There is no significant difference between normal condition and oxidative stress condition in *nox1Δ* mutant. Together, *CgNOX1* is associated with both the oxidative stress response and a hepatic infection model.

Chapter 3 The effect of *CgNOX1* deficiency on inflammation response and NLRP3 induction in hepatocytes following *C. glabrata* infection.

In the previous chapter, I explored the influence of hepatocytes on the CgNOX1 gene. Therefore, in this chapter, my focus shifts to investigating the reverse effect within this model.

My results indicated that the inflammatory response genes (TNF-α, IL-1β and IL-6) were induced in HC cells after *C. glabrata* infection. The lack of *CgNOX1* reduced the induction of these genes in transcript level.

During the co-incubation, the cytokine IL-6 in hepatocytes was markedly induced in wild-type stain, both at transcriptional level and protein level.

However, TNF-α and IL-1β cytokines were not detected in HC cells after *C. glabrata* infection with LPS priming by ELISA. Moreover, NLRP3 inflammasomes and related genes (*Caspase-1, GSDMD, IL-18, ASC* and *NF-κB*) were not induced in hepatic cells after *C. glabrata* infection.

Chapter 4 GFP expression driven by *CgNOX1* promoter during co- incubation with hepatic cells and under oxidative stress.

In this chapter, I investigated the regulation of *CgNOX1* promoter during co-incubation with hepatocytes and under oxidative stress by using a green fluorescent protein (GFP) system. Using GFP reporter constructs, the expression levels driven by *CgNOX1* promoter by monitoring GFP expression by confocal microscopy or by RT-qPCR were analyzed under various conditions.

First, a GFP reporter system was established, consisting of *CgNOX1* promoter individually to investigate the promoter's activity. However, only faint GFP signals were detectable in some certain cells, while not all cells displayed fluorescence. Moreover, there is no fluorescence signal difference between WT strain and GFP-tagged strain after the co-incubation with HC cells or treatment with H_2O_2.

Chapter 5 GFP expression driven by *CaFRE8* promoter during co- incubation with hepatic cells and under oxidative stress.

In *C. albicans*, Fre8, a member of NOX family, has been reported to produce a burst of ROS and be identified as a key factor in this fungus for hepatic TG2 induction in the co-cultures. Therefore, it is of great significance to elucidate the transcriptional regulation mechanism of *CaFRE8* gene during co-incubation with hepatocytes and under oxidative stress. In this chapter, I developed *C. albicans* strains containing *CaFRE8* promoter and GFP as a reporter gene, following the methods from the previous chapter.

At first, *C. albicans FRE8Δ/yEGFP-CYC1t* did not induce GFP signals in the presence or absence of HC cells and hydrogen peroxide. However, after the insertion of *CaACT1* promoter, a strong promoter, the *C. albicans FRE8Δ/yEGFP-ACT1pt-CYC1t* exhibited detectable GFP fluorescence by both confocal microscopy and flow cytometry. Furthermore, under the oxidative stress and co-incubation with HC cells, the GFP signals became significantly higher, which is consistent with the transcript expression

results. These findings suggest that this strain could serve as a suitable model for quantitative analysis of the *FRE8* promoter.

Chapter 6 Functional analysis of the *Candida albicans FRE8* promoter

In this chapter, a functional analysis of the intergenic region upstream (-500 bp to the ATG start codon) of the *CaFRE8* gene was conducted. To identify the core regulatory region of the promoter in *C. albicans*, a series of GFP-tagged mutants containing five promoter deletion fragments were constructed. The constructed recombinants were then utilized to monitor GFP signals using the previous methods under the specific conditions.

At first, I established five GFP-tagged *C. albicans* strains, each with a different deletion of the *CaFRE8* promoter region spanning 500 bp upstream of the gene. The GFP fluorescence of all the GFP-tagged strains was detectable and showed no meaningful difference.

Then, during the co-incubation with hepatic cells, it was determined that the region from -500 bp to -401 bp in the upstream sequence of the gene is considered as a core region. In addition, it was observed that the -99 ~ 0 region of the *CaFRE8* promoter might play a critical role under conditions of oxidative stress.

Chapter 7 Conclusion

In conclusion, *CgNOX1* plays an important role in *C. glabrata* during the co-incubation with human hepatocytes and under oxidative stress.

Furthermore, in *C. albicans,* the core regions in the upstream sequences of the *CaFRE8* gene were identified during the co-incubation with HC cells and under oxidative stress.

The identification of the core sequences in *CaFRE8* upstream region might contribute to a better understanding of the gene induction mechanisms in *C. albicans*.