

令和 6 年 6 月 5 日現在

機関番号：12608

研究種目：若手研究

研究期間：2021～2023

課題番号：21K15431

研究課題名（和文）Investigation on the induction mechanism of hepatocyte death by fungal reactive oxygen species

研究課題名（英文）Investigation on the induction mechanism of hepatocyte death by fungal reactive oxygen species

研究代表者

Chen Xinyue (Chen, Xinyue)

東京工業大学・生命理工学院・助教

研究者番号：50817369

交付決定額（研究期間全体）：（直接経費） 3,500,000 円

研究成果の概要（和文）：本研究では、カンジダ属菌に由来する 2 つの NADPH オキシダーゼが真菌と肝細胞の間の相互作用、および ROS 応答にどのように寄与しているかを調べた。C. glabrata の CgNOX1 遺伝子が真菌の ROS 産生、酸化ストレスへの応答、鉄還元酵素活性において重要な役割を果たしていることを実証した。Candida albicans では、ROS 産生に関わる CaCFL11 について、GFP レポーターを使用し、共培養および酸化ストレス条件下で CaCFL11 プロモーターの解析を行った。-200 bp から開始コドンまでの上流領域が転写制御に必要なコアプロモーター領域であることを示唆した。

研究成果の学術的意義や社会的意義

本研究は、カンジダ属菌に由来する活性酸素 ROS 産生酵素 NADPH オキシダーゼ遺伝子の機能および肝細胞との共培養時の遺伝子発現誘導を理解するための基礎研究である。真菌 ROS 産生を介して肝細胞の細胞死を誘導する分子メカニズムを理解することで、肝臓さらに全身性カンジダ症に対する臨床治療法の確立につながると考えている。真菌 ROS 産生を抑制することができれば、肝細胞の細胞死を防ぐことができ、カンジダ属菌が腸管から肝臓、さらには全身への侵襲を食い止めることができるのではないかと期待される。

研究成果の概要（英文）：This study investigated how two NADPH oxidase genes from Candida species contribute to the interplay between fungi and hepatocytes and involve in oxidative stress responses. Our results demonstrated CgNOX1 in Candida glabrata plays important roles in fungal ROS production, the response to oxidative stress and ferric reductase activity as well as affects the production of inflammatory cytokines in hepatocytes following C. glabrata infection. In Candida albicans, CaCFL11 showed enhanced expression under the co-culture with hepatocytes, resulting in ROS production. By using a GFP reporter system, the regulation of CaCFL11 promoters was studied during co-incubation with hepatocytes and under oxidative stress. Our results suggested CaCFL11 upstream region from -200 bp to the start codon was essential for transcriptional regulation.

研究分野：life science

キーワード：Candida species NADPH oxidase fungal ROS production

1 . 研究開始当初の背景

Pathogenic *Candida* species which are the most common causes of invasive fungal infection worldwide can invade gastrointestinal mucosa and reach liver to cause serious systemic inflammation. By co-incubating the *Candida* cells with human hepatic cell line (HC cells), the induction of nuclear transglutaminase (TG) activity in HC cells has been reported to result in hepatocyte death. Therefore, understanding the interaction between *Candida* virulence factor and host response is of great importance. Our previous studies showed the expression levels of NADPH oxidases (Nox) genes were evaluated in *Candida* species, which resulted in the generation of fungal ROS after co-incubation with HC cells. These suggested that generation of ROS is one *Candida* virulence factor which is responsible for the induction of hepatocyte death. However, the detail functions of fungal Nox during infection and processes about how do the fungal ROS effect on the induction of hepatocyte death are still unknown.

2 . 研究の目的

The aim of this study is to explore the molecular mechanism of fungal ROS production during infection on human hepatic cells, by identifying the roles of *Candida NOX* genes in fungal ROS production and the responses by hepatic immune systems.

3 . 研究の方法

The expression levels of *NOX* family genes were screened by real-time PCR when co-incubated with HC cells and single incubation, respectively. The genes whose expression are significantly enhanced during co-incubation with HC cells were investigated in further research. By using these gene disrupted mutants, the roles of these genes were studied during fungal ROS production. Green fluorescent protein (GFP) was tagged as a reporter gene to identify the transcription factors which regulate the transcription of *NOX* genes in *Candida* species during infection. The relative experiments were conducted by fluorescence intensity detection and confocal microscopy assays. To study the responses of hepatic immune system, the expression levels of relative cytokines in HC cells were detected when co-incubated with fungi, comparing with single incubation. The protein levels were confirmed by western blotting and ELISA assays. Antifungal susceptibility test was also performed to study the effect of natural product curcumin on *C. albicans*.

4 . 研究成果

This study investigated how two NADPH oxidase genes from *Candida* species contribute to the interplay between fungi and hepatocytes and involve in oxidative stress responses. Our results demonstrated *CgNOX1* in *Candida glabrata* plays important roles in fungal ROS production, the response to oxidative stress and ferric reductase activity as well as affects the production of

inflammatory cytokines in hepatocytes following *C. glabrata* infection. It also contributes to the induction of host TG activity during co-incubation with human hepatic cells and the fungus, which may be mediated by the production of fungal ROS.

In *Candida albicans*, *CaCFL11* showed enhanced expression under the co-culture with hepatocytes, resulting in ROS production. It plays a crucial role during co-incubation with hepatocytes and under oxidative stress response. By using a GFP reporter system, the regulation of *CaCFL11* promoters was studied during co-incubation with hepatocytes and under oxidative stress. Our results suggested *CaCFL11* upstream region from -200 bp to the start codon was essential for transcriptional regulation.

On the other hand, our study on antifungal drug resistance showed the natural product curcumin affected the function of heat shock protein 90 (Hsp90) and drug efflux pump activity of multidrug transporter protein Cdr1 in *C. albicans*.

5. 主な発表論文等

〔雑誌論文〕 計2件（うち査読付論文 2件／うち国際共著 2件／うちオープンアクセス 2件）

1. 著者名 Yean Sheng Lee, Xinyue Chen, Tria Widiastih Widiyanto, Kanami Orihara, Hiroyuki Shibata and Susumu Kajiwara	4. 巻 12
2. 論文標題 Curcumin affects function of Hsp90 and drug efflux pump of <i>Candida albicans</i>	5. 発行年 2022年
3. 雑誌名 Frontiers in Cellular and Infection Microbiology	6. 最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fcimb.2022.944611	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

1. 著者名 Maoyi Lin, Yao Huang, Kanami Orihara, Hiroji Chibana, Susumu Kajiwara and Xinyue Chen	4. 巻 10
2. 論文標題 A Putative NADPH Oxidase Gene in Unicellular Pathogenic <i>Candida glabrata</i> Is Required for Fungal ROS Production and Oxidative Stress Response	5. 発行年 2023年
3. 雑誌名 Journal of Fungi	6. 最初と最後の頁 16
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/jof10010016	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕 計2件（うち招待講演 0件／うち国際学会 1件）

1. 発表者名 Maoyi Lin, Maho Shirai, Xinyue Chen, Kanami Orihara, Susumu Kajiwara
2. 発表標題 Analysis of hepatocyte apoptosis induction mechanism by pathogenic fungus <i>Candida</i> species
3. 学会等名 The 95th Annual Meeting of the Japanese Biochemical Society
4. 発表年 2022年

1. 発表者名 Xinyue Chen, Yao Huang, Hiroji Chibana, Kanami Orihara, Susumu Kajiwara
2. 発表標題 Roles of a putative NADPH oxidase gene in <i>Candida glabrata</i>
3. 学会等名 19th Asia Pacific Congress of Clinical Microbiology and Infection（国際学会）
4. 発表年 2023年

〔図書〕 計0件

〔産業財産権〕

〔その他〕

-

6. 研究組織

	氏名 (ローマ字氏名) (研究者番号)	所属研究機関・部局・職 (機関番号)	備考
--	---------------------------	-----------------------	----

7. 科研費を使用して開催した国際研究集会

〔国際研究集会〕 計0件

8. 本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関
---------	---------