論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	Fungal ROS generation mechanism for induction of transglutaminase 2 activity in human hepatocyte
著者(和文)	HuangYao
Author(English)	Yao Huang
出典(和文)	学位:博士(理学), 学位授与機関:東京工業大学, 報告番号:甲第11336号, 授与年月日:2019年12月31日, 学位の種別:課程博士, 審査員:折原 芳波,一瀬 宏,長田 俊哉,小畠 英理,小倉 俊一郎,梶原 将
Citation(English)	Degree:Doctor (Science), Conferring organization: Tokyo Institute of Technology, Report number:甲第11336号, Conferred date:2019/12/31, Degree Type:Course doctor, Examiner:,,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

専攻： Department of	分子生命科学　専攻	申請学位（専攻分野）： Academic Degree Requested	博士（　理学　） Doctor of
学生氏名： Student's Name	Huang Yao	指導教員（主）： Academic Supervisor(main)	梶原　将
		指導教員（副）： Academic Supervisor(sub)	

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Candida glabrata is a haploid yeast of genus *Candida*. It is an opportunistic fungi and causes candidiasis. It is the most common fungal pathogen of human digestive tract, especially prevalent in HIV positive people, and the elderly. The ability of *Candida* species to infect such diverse host is associated by various virulence factors such as proteinases and phospholipases.

NADPH oxidases (Nox) generate reactive oxygen species (ROS) such as superoxide anion radical (O_2^-) and hydrogen peroxide (H_2O_2). ROS generation naturally takes place as bi-production of cellular aerobic metabolism and biosynthesis by ROS-generating enzymes. Mitochondrial respiration is thought to provide the main source of ROS in eukaryotic cells via the process of oxidative phosphorylation. Leakage of electrons from the respiratory chain results in the reduction of oxygen and sometimes generating ROS in the cells. ROS are also generated by xanthine oxidase and NADPH oxidase. Xanthine oxidase is one of catalytic enzymes for purines and produces superoxide. Nox superfamily is known to be membrane-located enzyme catalyzing from NADPH and oxygen to superoxide. A possible function of *Podospora anserina* Nox was indicated by the recent observation on the mutation of *PRO41*, encoding a homolog of an ER protein in *Sordaria macrospora*. Also a Nox ortholog in *S. cerevisiae,* Yno1p (Aim14p), was shown to be mainly localized in ER membranes. From these findings, it is suggested that fungal Nox is located in the ER. Then ER is also thought to be a significant organelle for ROS production in fungi.

Transglutaminases (TG) is a group of enzymes that catalyze the formation of an iso-peptide bond between a free amine group (e.g., protein- or peptide-bound lysine) and an acyl group at the end of the side chain of protein- or peptide-bound glutamine. Transglutaminase 2 (TG2) is the most abundantly expressed member of the TG family. TG2 is a Ca^{2+}-dependent formation of a covalent bond between γ-carboxamide groups of peptide-bound glutamine residues and the primary amino groups of a wide range of proteins. This crosslinking activity has been implicated in various physiological activities such as cell growth and differentiation, cell adhesion and apoptosis. In low intracellular Ca^{2+} concentrations as 10 nM, ATP/GTP/GDP forms complex with TG2 and render TG2 in a “closed” conformation. In this conformation, it mainly acts as a GTPase supporting growth. In contrast, when cells are injured and intracellular Ca^{2+} concentration exceeds 700–800 nM, Ca^{2+} binds with TG2 to make an extended “open” conformation and exposing the catalytic site of TG2. The “open” conformation of TG2 mainly acts as a crosslinking enzyme and induces

apoptosis. TG2 is mostly a cytosolic protein but also can localize in extracellular matrix, the membrane, mitochondria and nucleus. Only small portions (5-7 %) are expressed in the nuclear location, which is generally stimulated by cellular stresses such as ROS and an increased Ca^{2+} concentration. Because of the cellular location and biological activity of TG2, this protein induces cell growth, differentiation and death.

TG2-dependent hepatic cell death has been previously reported in alcohol, free fatty acid, and acyclic retinoid (ACR) treated cell culture systems, animal models and patients with ASH and NASH. In hepatic cells, treatment with alcohol and free fatty acid increases crosslinking activity of TG2 in nucleus. The increased nuclear TG2 activity causes excessive crosslinking of transcription factor Sp1 and inactivates it. This inactivation decreases expression of c-Met, one of major receptors for hepatocyte growth factors, leading to hepatic cell death.

Recently, it was reported that pathogenic fungi *C. glabrata* enhanced cellular transglutaminase 2 (TG2) activity in human hepatic cells mediated by ROS. A deletion of *C. glabrata NOX1* gene (*CgNOX1*) has shown to fail to induce the TG2 activity. However, the effect of fungal ROS to human cells has not been understood in detail. In this work I investigated the role of fungal *NOX* genes under the co-incubation with human hepatocyte. I found that fungal *NOX* genes contribute to the generation of extracellular ROS and large amount of fungal ROS influences the TG2 activity in hepatocyte. *Cg*Nox1p has an important rule to induce TG2 activity in hepatic cells and *CgNOX1* gene was induced when *C. glabrata* was co-incubated with HC cells. On the other hand, *C. glabrata FRE6* (*CgFRE6*) gene was identified as other *NOX* gene and also induced when co-incubation with HC cells. However, the *fre6* mutant of *C. glabrata* was capable of inducing TG2 activity in hepatocytes as same as the wild type. Therefore, although *Cg*Fre6p can produce exogenous ROS, it was suggested that ROS yield form *Cg*Fre6p was not enough to induce TG2 activity in HC cells. In addition, overexpression of *YNO1* gene, which is a homolog to *CgNOX1*, in *Saccharomyces cerevisiae* led to induction of ROS generation and TG2 activity in HC cells when this transformant was co-incubated with the hepatic cells. These findings indicated that, at least, a fungal Nox plays a role in enhancing TG2 activity in human hepatocytes and leads to the apoptosis.