論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	The molecular biological study for functional expression of exogenous ABC transporters in the genome-editted Saccharomyces cerevisiae
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出典(和文)	学位:博士（学術）, 学位授与機関:東京工業大学, 報告番号:甲第10360号, 授与年月日:2016年9月20日, 学位の種別:課程博士, 審査員:梶原 将,長田 俊哉,林 宣宏,小畠 英理,小倉 俊一郎
Citation(English)	Degree:, Conferring organization: Tokyo Institute of Technology, Report number:甲第10360号, Conferred date:2016/9/20, 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

（論文博士）

論　文　要　旨（英文）　（800語程度）

（Summary）

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（要　旨）

Many ABC transporters work as drug efflux pumps in eukaryotic cells and the over-expression of these proteins lead to the multidrug resistance of pathogens and cancer cells. Thereby, it's important to clarify the function of these ABC transporters for the new drugs development. In this study, for the establishment of effective functional expression system for human ABC transporters, *Saccharomyces cerevisiae* ADΔ expression system was used. Although yeast is eukaryotic microorganism, its membrane structure and protein traffic system are different to those of human. Human plasma membrane (PM) consists of cholesterol as main membrane sterol whereas yeast PM contains ergosterol. Therefore, construction of cholesterol-producing *S. cerevisiae* strain as the host and improvement of protein trafficking and endocytosis in this yeast for human ABC transporter were examined. On the other hand, other yeast species such as *Candida utilis* have membrane components more similar to human PM. In order to develop an alternative host, all ABC proteins of *C. utilis* were identified.

Endocytosis is the process allowed the cells to removal unwanted membrane protein and nutrition uptake. Yeast, unlike mammalian cell, requires ubiquitination as initial step for

endocytosis. Mutation in each step of ubiquitination machinery can resulted in decrease internalization and stabilized the proteins on PM. Most of the yeast PM protein requires HECT ubiquitin ligase Rsp5p/Npi1p which deletion of the Rsp5p C2 domain delayed down-regulate of Gap1p, a general amino acid permease as well as stabilized Fur4p, uracil permease on the cell PM. Yeast ADΔRsp5ΔC2 strain was further used for expressing human ABCB1 transporter. The analysis to determine improvements in function and/or localization of those transporters were conducted by disk susceptibility assay, protein expression and intact cell fluorescence detection. All the results, suggested C2 domain deleted strain might not suitable to improve *Hs*ABCB1 function. However, it can be confirmed that Rsp5ΔC2 constructed in this work, was function by expression of *S. cerevisiae* uracil permease, Fur4p in ADΔRsp5ΔC2 strain compared with expression in ADΔ. The C2 domain manipulation improved Fur4p function by increase uracil uptake into the cell than that Fur4p expressed in ADΔ with wt Rsp5p.

Biochemical studies of *Hs*ABCB1 found that lipid environment in cell membrane dramatically affect ATPase activity and also supported by a studies in cholesterol depletion cell line shown reduction in transporter activity and led to intracellular drugs accumulation. Cholesterol is a major sterol component of mammalian cell membranes becomes an important factor for *Hs*ABCB1 function while the major sterol in yeast PM is ergosterol. Expression of ABCB1 in *S. cerevisiae* reported a significantly decreased drug binding activity when compared to native protein. I constructed yeast cholesterol producing strains ADΔ57624 with TDH3 or ADH1

overexpression promoter and confirmed its cholesterol production by Gas-chromatography. The sterol productions in these strains were shown as sterol mixture of desmosterol and cholesta-5, 7, 24-trienol along with small cholesterol detected. However, this strain was further used for *Hs*ABCB1 expression and evaluated its functional expression by disk susceptibility assay. Unfortunately, there have no significant improvement of *Hs*ABCB1 in both function and localization in this strain.

Candida utilis is becomes an interesting candidate for developing as an alternative host for the expression of membrane proteins such as ABC transporters for functional analysis. In this study I identified all putative *C. utilis* ABC proteins. In *C. utilis* WGS was predicted to have 30 ABC protein-encoding ORFs belonging to six of the seven major ABC subfamilies. In short, all 30 ABC proteins in *C. utlis* are in ABCB (1 full-size plasma membrane and 3 mitochondrial half-size ABC transporters), ABCC (5 full size PM ABC transporters and 3 vacuole full-size ABC transporters), ABCD (2peroxisomal half-size ABC transporters), ABCE (1 ABC protein) ABCF (4 ABC proteins) and ABCG (8 full sized PM ABC transporters and 1 unique ABC transporters). Other two of these 30 ABC proteins, homologs of *S. cerevisiae* YDR061w and CAF16, could not be classified as belonging to any ABC subfamilies, instead they appear to be fungal-specific ABC proteins.

One ORF, which we named *C. utilis CDR1* (*CuCDR1*), encoded a protein with the most higher similarity to the well studies *C. albicans* multidrug efflux pump Cdr1p. Overexpression of

CuCDR1 in the genome-edited *S. cerevisiae* host ADΔ led to a functional protein and high levels of resistance to many xenobiotics very similar to *Ca*Cdr1p. However, a GFP-tagged which fused directly to the C-terminus of *Cu*Cdr1p did not express well and was not functional; this problem that could be rectified by engineering a 15 amino acid linker-GAGGSAGGSGGAGAG- between *Cu*Cdr1p and GFP or by placing the GFP-tag at the N-terminus of *Cu*Cdr1p. We conclude that *Cu*Cdr1p is a multidrug efflux pump, the overexpression of which may cause multidrug resistance of opportunistic *C. utilis* infections in the future, and deletion of *CuCDR1* may be advantageous for the construction of a host suitable for the heterologous expression of membrane transporters.