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A critical role for very long-chain fatty acid elongases in oleic acid-mediated *Saccharomyces cerevisiae* cytotoxicity

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ABSTRACT

Elongases FEN1/ELO2 and SUR4/ELO3 are important enzymes involved in the elongation of long-chain fatty acids (LCFAs) to very long-chain fatty acids (VLCFAs) in *Saccharomyces cerevisiae*. The molecular mechanism of the involvement of these elongases in lipotoxicity is unclear. In the present study, we investigated the role of VLCFA elongases in oleic acid-mediated yeast cytotoxicity. The spot test showed that yeast strains with the deletion of *ELO2* or *ELO3* were strikingly sensitive to oleic acid, while there was no change on the growth of strain with deleted *ELO1* which was involved in the elongation of C_{14} fatty acid (FA) to C_{16} FA. By using GC–MS, the unsaturation index was increased in *elo2*$^{\Delta}$ and *elo3*$^{\Delta}$ mutants after treatment with oleic acid (OLA). However, the proportion of VLCFAs was increased in response to OLA in the wild-type strain. The growth inhibition of *elo2*$^{\Delta}$ and *elo3*$^{\Delta}$ could be partially rescued by two commonly used antioxidant agents N-acetyl cysteine (NAC) and Ascorbic acid (VC). The further study showed that exposure to excess OLA led to an increase in the levels of reactive oxygen species (ROS) and thiobarbituric acid reactive substances (TBARS), and a decline in the quantity of reduced glutathione (GSH) in both the wild type and mutant strains. However, the antioxidant enzyme activities of superoxide dismutase (SOD) and catalase (CAT) were increased in the wild type and *elo1*$^{\Delta}$ strains, while they were significantly decreased in the mutants of *elo2*$^{\Delta}$ and *elo3*$^{\Delta}$ after treated with excess OLA. Thus, oxidative damage mainly contributed to the cell death induced by OLA in *ole2*$^{\Delta}$ and *ole3*$^{\Delta}$. Taken together, although disruption of *ELO2* or *ELO3* did not affect the cellular lipid unsaturation, they altered the distribution and propotion of cellular VLCFAs, leading to the cell membrane impairment, which augmented the ability of OLA to permeabilize the plasma membrane. The data suggest that the very long-chain fatty acids elongases *ELO2* and *ELO3* play important roles in lipotoxic cell death induced by OLA through maintaining a balanced FA composition in plasma membrane.

1. Introduction

Fatty acids (FAs) are main components of cellular lipids, including glycerolipids, sphingolipids, and cholesterol esters, and the imbalance of their metabolism is closely related to cell dysfunction. Excess FAs accumulation in non-adipose tissues is a hallmark of metabolic disease (Brookheart et al., 2009). Lipotoxicity is the pathological consequence of lipid overflow in non-adipose tissue, mediated through reactive lipid moieties which may even lead to lipid-induced cell death (Kohlwein, 2010). Nevertheless, the underlying mechanism that results in

Abbreviations: FA, fatty acid; CAT, catalase; GP_X, glutathione peroxidase; GSH, glutathione; MDA, methane dicarboxylic aldehyde; NAC, N-acetyl cysteine; OLA, oleic acid; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; UFA, unsaturated fatty acid; VC, ascorbic acid; VLCFA, very long chain fatty acid

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lipoapoptosis is largely unknown. The yeast mutants (*ARE1*, *ARE2*, *LRO1,* and *DGA1*) lack detectable neutral lipids and cytoplasmic lipid droplets. Moreover, they are also hypersensitive to unsaturated FAs oleic acid (C18:1, OLA) and palmitoleic acid (C16:1). By contrast, the saturated FAs palmitic acid (C16:0) and stearic acid (C18:0) had no detectable impact on the viability of these strains (Garbarino et al., 2009). Lockshon et al. identified numerous yeast mutants that are hypersensitive to unsaturated OLA treatment, including mutants lack of peroxisomal-oxidation. Thus, the increase of − permeability of plasma membrane was the main reason that lead to OLA inhibition of peroxisomal mutant (pex6) growth. It might result from the loss of the ability to effectively control the fatty acid composition of membrane phospholipids. Lockshon et al. also found that mutant lack of very-long-chain fatty acids (VLCFAs) enzyme *FEN1* showed OLA sensitivity and its growth was significantly inhibited by OLA, yet strains deleted in genes that encoded either of the other elongases, *ELO1* and *SUR4*, showed no OLA phenotype (Lockshon et al., 2007). Heat and oxidative stress may share a common mechanism of damage through induction of oxygen-derived free radicals, resulting in membrane lipid damage. The extent of cellular damage was related to membrane lipid composition and correlated positively with increasing unsaturation of the phospholipid fatty acyl component (Garbarino et al., 2009).

The FA elongation reactions and responsible enzymes are conserved among eukaryotes, and the yeast *Saccharomyces cerevisiae* has three FA elongases, which are ELO1, FEN1/ELO2 and SUR4/ELO3, respectively (Tehlivets et al., 2007).Among these three yeast FA elongases, FEN1 and SUR4 are involved in VLCFA synthesis (Oh et al., 1997; Rossler et al., 2003), and ELO1 is involved in the elongation of C14 FA to C16 FA (Tehlivets et al., 2007; Toke and Martin, 1996). The elongations of long-chain FAs or shorter VLCFAs to VLCFAs take place in endoplasmic reticulum. FAs elongation follows a four-step cycle (condensation, reduction, dehydration and reduction) to make the FAs carbon chain has two more carbons than precursor acyl CoA. (Tehlivets et al., 2007; Kihara, 2012). FEN1 and SUR4 can both catalyze the first condensation reaction, the rate-limiting step, and determine the length of the VLCFA products. SUR4 produces C26 VLCFA, whereas FEN1 is involved in the production of C24 or shorter VLCFAs (Oh et al., 1997; Denic and Weissman, 2007). Yeast cells carrying single deletion of *FEN1* or *SUR4* are viable due to some overlap in activities, while simultaneous disrupted mutants are inviable (Revardel et al., 1995).

VLCFAs have chain lengths from 20 to 30 carbons, or greater (Millar and Kunst, 1997). They are composed of sphingolipids and GPI-anchors that play essential roles in the yeast cell (Tehlivets et al., 2007; Kihara, 2012). They presumably serve specific structural functions. Defective fatty acid elongation has deep impacts on lipid (Schneiter et al., 1999) and protein sorting, as well as plasma membrane-localized H^+-ATPase to the plasma membrane (Gaigg et al., 2005). Mutants in *ELO2* were resistant to fenpropimorph, a drug affecting sterol metabolism (Lorenz and Parks, 1991). The *elo3* mutants could suppress a mutation in RVS167 (Revardel et al., 1995), which lead to reduced viability upon starvation. Additionally, both *elo2* and *elo3* mutants were identified as v-SNARE bypass mutants, *vbm2* and *vbm1* (David et al., 1998). VLCFAs also play a key role in membrane and cytoskeletal functions (Silve et al., 1996), as well as the maintenance of a functional nuclear envelope and the biogenesis of microautophagic vesicles (Schneiter et al., 1996; Lee et al., 2012).

In this study, we explored the lipotoxic of OLA on FA elongases deleted strains to address the molecular mechanisms that controlled FA fluxes. This helps to well understand the physiological consequences of a lipotoxic insult. We found that mutants defective in *ELO2* and *ELO3* were sensitive to OLA, while that did not occur in *elo1*△. Co-treatment of OLA and antioxidant agents NAC or VC reduced the lipotoxic cell death. We examined oxidative damage to cell structures and antioxidant system to find the causes of OLA toxic to these mutants. By the end, we found that *ELO2* and *ELO3* may participate in the maintenance of plasma membrane integrity.

2. Materials and methods

2.1. Yeast strains, plasmids, media and growth

Yeast strains used in this study include the wild-type haploid strain BY4741 (*MAT a his3*Δ1 *leu2*Δ0 *lys2*Δ0 *ura3*Δ0), and the related mutant strains, including *elo1*△ (BY4741 *elo1*: KanMX), *elo2*△ (BY4741 *elo2*: KanMX), *elo3*△ (BY4741 *elo3*: KanMX). Plasmids expressing *ELO1*, *ELO2* and *ELO3* from a centromere-based plasmid were constructed by *in vivo* homologous recombination into YCplac33 (CEN and URA3) (Gietz and Sugino, 1988). YCplac33 was used as the vector control.

Yeast strains were cultured in YPD (1% yeast extract, 2% peptone, and 2% dextrose with or without 1.5% agar) medium at 30 ˚C. OLA was stocked in ethanol before mixed with 50% NP-40. Control media contained 0.5% ethanol and 0.1% NP-40, which did not influence cell growth. For liquid cultures, cells were pre-cultured overnight, shifted to fresh medium, grown to log phase (OD_{600} = 0.6), and exposed to OLA at 30 ˚C, 180 rpm for 18 h. For growth tests, cells were adjusted to OD_{600} = 1.0, and serially diluted in 1:10 steps, and 3 μL of the respective dilutions were spotted onto media plates containing 1.5% agar.

2.2. Fatty acid analysis

FAs were extracted from approximately 100 OD_{600} units of yeast cells by two-step lipid extraction (Ejsing et al., 2009). And fatty acid methyl esters were performed by BF_3-methanolysis of whole cell lipids as described previously (Morrison and Smith, 1964). The fatty acid methyl esters were then dissolved in 200 μL of hexane and analyzed by gas chromatography–mass spectroscopy using an Agilent 6890N GC 5975C Inert MSD GCMS system (Agilent, USA). The injector temperature was 240 ˚C. The flow rate of helium gas through the column was 1 mL/min. The column temperature increased from 80 ˚C to 280 ˚C at a rate of 20 ˚C/min, and then held for 5 min. The identity of each peak in the chromatogram was confirmed by comparison with the mass spectra database.

2.3. ROS assay

ROS was measured with 2′,7′-dichlorofluorescein diacetate (DCFH-DA, Sigma-Aldrich, USA). Cells were collected by centrifugation at 5000 rpm for 5 min in 2-mL centrifuge tubes after cultured for 18 h. The pellets were washed twice with 50 mM ice-cold phosphate-buffered saline (PBS, pH 7.0) and resuspended in 1 mL of the same buffer. Then 1 μL of 10-mM DCFH-DA (stock in DMSO) were added and incubated at 37 ˚C for 30 min. Cells were subsequently washed twice with PBS, and resuspended in 200 μL PBS before shifted to 96-well plate. The DCFH fluorescence intensity of 10^7 cells was measured by an automatic microplate reader (Biotek, USA) using 488-nm excitation and 525-nm emission filters (Liu et al., 2013).

2.4. Determination of antioxidant enzyme activities

The activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GP_X) were assayed in the supernatant of cell lysate. CAT activity was determined by measuring the rate of H_2O_2 consumption after adding the supernatant to the mixture containing 50 mM potassium phosphate buffer (pH 7.0) and 10 mM H_2O_2 at 30 ˚C for 10 min at 240 nm (Aebi, 1984)with a UV-2100 UV/Vis spectrophotometer (UNIC, China). One unit of enzyme activity corresponded to decomposition of 1 μmole substrate during 1 min. GP_X activity was determined by a previously described procedure (Hafeman et al., 1974). SOD activity was determined by a previously described procedure as well (Distelbarth et al., 2013).