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Yeast cell death induced by high intensity ultrasound

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1. Introduction

Understanding of cell death caused by ultrasonic exposure is important factor in the field of ultrasound diagnosis and clinical trial[1]. Biological cell death is classified with three type: apoptotic cell death or programmed cell death, necrotic cell death and autophagic cell death. Each death form is defined as a morphological structure and specific death pathway. Recently, it was reported ultrasound irradiation could induce apoptotic cell death in humangastric carcinoma and leukemia cell lines.[2,3] However, the precise mechanism and efficacious method of ultrasound induced-cell death remains to be elucidated. In this study we investigated the ultrasonic irradiation induced cell death in yeast Saccharomyces cerevisiae. And to determine whether ultrasound-induced cell death were related to apoptosis or necrosis, we used Yca1, AIF1, Kex1, NHP6 deletion mutants. Furthermore, using GFP-Atg8 expression transformed yeast cell strain, we investigated the possibility of autophagy caused by various ultrasound irradiation conditions.

2. Materials and methods

2.1 Yeast Cell Culture

Yeast Saccharomyces cerevisiae BY4743 and mutant strains were purchased from the EUROSCARF. Yeasts strains are incubated in YPD or in defined synthetic medium at 30 °C. Ultrasonic irradiation with various conditions were exposed to yeast cell culture according to Fig. 2.

2.2 Ultrasound Irradiation System

A pyrex beaker with 60 mm OD was used as an ultrasonic irradiation cell. BLT (Bolt-clamped Langevin Transducer) with 40 kHz nominal frequency was attached at the bottom of irradiation cell. BLT was excited by an ultrasonic generator (50W, Kodo Technical Research Co., Korea). Distilled water of 20 mm height, which is approximately half wavelength of 40 kHz in water, was filled into irradiation cell, so that violent cavitation occurred. A pyrex tube with flat bottom of 12 mm ID, in which yeast culture is contained, is located on the surface of the water in the ultrasonic irradiation cell. [5].

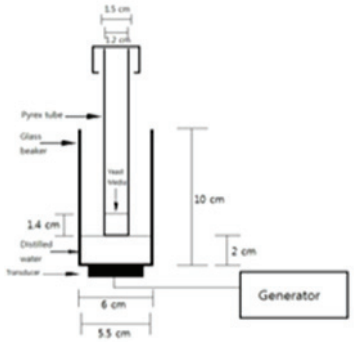

Fig.1 Schematic diagram of ultrasound irradiation.

2.3 Cell death assay

Ultrasonic waves at a frequency of 40.9 kHz were irradiated to 1.7 ml of culture with different time intervals. To measure the viability of ultrasonic irradiation, we used colony formation assay, proliferation assay or DAPI staining. For observing the ultrasound induced autophagy in yeast, we used yeast strains expressing GFP-Atg8 were transformed with the plasmid pRS316-GFPAtg8p (kindly provided by Dr Yoshinori Oshumi, Japan). We used spermidine (4mM) treatment for positive control of autophagy. After ultrasonic irradiation, prepared cells were visualized on a Zeiss Axiovert40 fluorescence microscope (Zeiss).

3. Result

To determine optimal cell death with 40 kHz ultrasound irradiation, we examined cell viability after different ultrasonic exposure. Fig. 2 and Fig. 3 show a condition of a time dependent experiment for ultrasound irradiation on yeast cell line BY4743. Viability of cultured yeast cells decreased with increasing (1, 5 and 10 min) ultrasonic irradiation. To study whether ultrasound induced cell death is accompanied with DNA condensation, we stained ultrasonic irradiated yeast cells with DAPI (1μg/ml). Nuclear condensation was observed ultrasonic irradiated yeast cells. (Fig. 4) To determine whether

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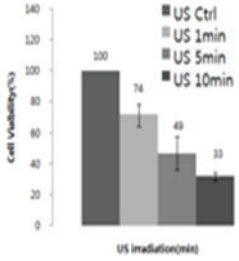

Fig2 Effect of ultrasound on the cell viability

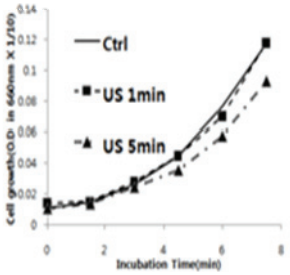

Fig3 Cell growth after ultrasound irradiation.

ultrasound irradiation induces the autophagy, We prepared yeast strains expressing GFP-Atg8 were transformed with the plasmid pRS316-GFPAtg8p. Using this strain, we analyzed autophagic granules after spermidine (4mM) treatment and/or ultrasound irradiation. (Fig. 5) To ask which death pathway was involved in ultrasound induced cell death, we analyzed the cell death pattern with mutant strains (ΔYca1, ΔAif1, ΔCdc42, ΔKex1, ΔNhp6)[5]. Cell viabilities of ΔAif1 and ΔKex1 are marked than other mutants (Fig 6).

Fig.4) Ultrasound could induce the nuclear condensation in yeast cells.

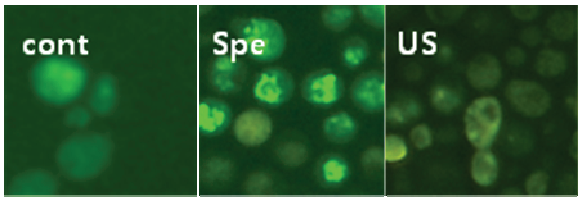

Fig.5) Ultrasound could not induce the autophagy compared to that of spermidine treatment.

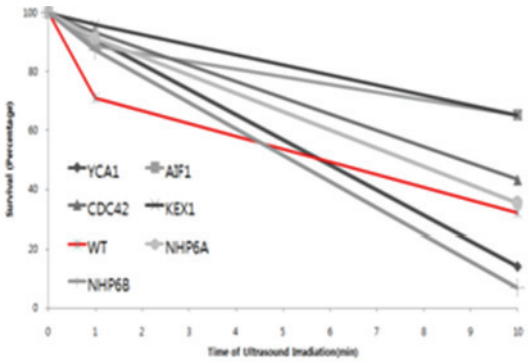

Fig.6) Deletion mutants (Aif1 and Kex1) were resistant to ultrasound-induced cell death.

4. Discussion

In this study, we investigated the ultrasound-induced cell death in yeast cell suspension. 40 kHz ultrasound irradiation increased yeast cell viability with increasing exposure time (up to 10 min, Fig. 2 and 3). As cavitation bubble formation in ultrasound system used in our study was observed, cell death might be caused in part by physical cavitation. To determine death pattern of ultrasound induced cell death, we investigated the nuclear condensation for apoptotic cell death (Fig 4), the Atg8 granule formation for autophagic cell death (Fig 5). We could observe the nuclear condensation of ultrasound irradiated yeast cell (Fig 4). However, we failed to observe the autophagy after ultrasound exposure which is sufficient to induce cell death (Fig 5). These results demonstrated that ultrasound could induce apoptotic cell death, but not autophagic cell death. Furthermore, we tried to investigate which death pathway is involved in ultrasound induced cell death. To do this, we selected death gene deletion mutants in yeast: Yca1 for caspase dependent death pathway, Aif1 for caspase independent death pathway and Nhp6A and B for necrotic death pathway. Fig.6 shows ΔAif1 and ΔKex1 mutants are strikingly resistant to ultrasound induced cell death. This results suggest ultrasonic irradiation may induce the apoptotic cell death via caspase-independent manner.

5. References

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