Daily intake of polyamine-rich *Saccharomyces cerevisiae* S631 prevents osteoclastic activation and bone loss in ovariectomized mice

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Abstract An imbalance in the sophisticated regulation between bone-resorbing osteoclasts and bone-forming osteoblasts leads to the pathogenesis and etiology of certain metabolic bone diseases including osteoporosis. Certain polyamines are related to the pathophysiology of some disorders, including Alzheimer's disease, infectious disease, cancer, and aging. Recently, we demonstrated that oral intake of polyamines (spermidine and spermine) prevented bone loss through preferential disturbance of osteoclastic

activation in ovariectomy-induced mouse model of postmenopausal osteoporosis. Here, we showed that daily oral supplementation of a diet containing polyamine-rich *Saccharomyces cerevisiae* S631 significantly inhibited osteoclastic activation as well as reduction of bone volume in the cancellous bone without affecting uterine weight in ovariectomized mice. Our findings recommend that daily oral supplementation with polyamine-rich yeast diet would be beneficial for prophylaxis of metabolic bone diseases associated with abnormal osteoclast activation.

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Introduction

Bone homeostasis is intricately modulated by bone-resorbing osteoclasts and bone-forming osteoblasts (Wagner and Karsenty, 2001). Osteoclasts are multinucleated cells derived from hematopoietic stem cells, whereas the osteoblast lineage is derived from multipotent mesenchymal stem cells (Chen et al., 2018). Imbalance between regulation by osteoclasts and that by osteoblasts results in metabolic bone diseases, such as osteoporosis or osteopetrosis (Feng and McDonald, 2011). Naturally occurring polyamines are indispensable to various cellular processes, including cellular viability, growth, and differentiation (Igarashi and Kashiwagi, 2010; Weiger and Hermann, 2014). These polyamines include putrescine (1,4-butane diamine), spermidine (*N*-(3-(aminopropyl)-1,4-butane diamine), and spermine (*N,N'*-bis (3-aminopropyl)-1,4-butane diamine).

We have previously demonstrated that spermidine and spermine, among the naturally occurring polyamines,

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prevent bone loss in ovariectomized mice through preferential disturbance of osteoclastic activation (Yamamoto et al., 2012). Based on our findings, we proposed that appropriate long-term consumption of a diet high in polyamine (spermidine and spermine) content would benefit the maintenance of bone health and the prophylaxis of menopausal osteoporosis. We recently isolated a novel *Saccharomyces cerevisiae* (*S. cerevisiae*) S631 strain with high concentration of polyamines by ultraviolet light exposure of commercially available baker's yeast (*S. cerevisiae*). In the current study, we attempted to demonstrate whether oral supplementation of a diet containing *S. cerevisiae* S631 with high concentration of polyamines could have a beneficial effect on ovariectomy (OVX)-induced mouse model that mimics postmenopausal osteoporosis.

Materials and methods

Isolation of *S. cerevisiae* S631 strain with high concentration of polyamines

For isolation of *S. cerevisiae* S631 strain with high concentration of polyamines, commercially available baker's

yeast (*S. cerevisiae*) was subject to ultraviolet light (UV) exposure and inoculation on agar media containing 3 mM spermine. The isolated strain was then subject to UV exposure and inoculation on agar media containing 6 mM spermine. A dansyl chloride-high performance liquid chromatography (HPLC) method was used for determination of the concentration of polyamines in *S. cerevisiae* S631 (Hunter, 1998).

Mice

The study protocol employed was in accordance with the guidelines of the Japanese Society for Pharmacology and was approved by the Committee for Ethical Use of Experimental Animals at the Kanazawa University. Female ddY mice were maintained in cages under a standard 12:12 h light/dark cycle with ad libitum access to food and water. Eight week-old mice were subjected to OVX or sham operation under aseptic environments, as previously described (Hinoi et al., 2007). Ovariectomized mice and sham-operated mice received daily oral supplementation of a diet containing 5% heat-sterilized *S. cerevisiae* S631 with high concentration of polyamines or normal chow. A dansyl chloride-HPLC method was used for determination

Fig. 1 Oral supplementation of diet containing high-polyamine yeast does not change diet intake, water intake and body weight. Eight-week-old female ddY mice were subjected to OVX, followed by daily oral supplementation of a diet containing 5% *S. cerevisiae* S631 with high concentration of polyamines for 28 consecutive days (n = 10).

Quantitative analyses were done with determinations of (A) diet intake, (B) water intake, (C) body weight and (D) uterine weight, respectively. ** $p < 0.01$, significantly different from each control value obtained in sham-operated mice

of the concentration of polyamines in diet (Hunter, 1998). Ovariectomized mice were also subjected to the intraperitoneal injection of 17β -estradiol (β E2), dissolved in corn oil, once a week for 28 consecutive days at a dose of $5 \mu\text{g/kg}$. The mice were killed by decapitation at 28 days after the operation, followed by dissection of their vertebrae, and subsequent fixation with 10% formalin.

Bone histomorphometric analyses

Bone histomorphometric analyses of the mice were performed using their undecalcified vertebrae, as previously described (Fukasawa et al., 2016). Briefly, the vertebrae were fixed with 10% formalin, dehydrated in different concentrations of ethanol, and subsequently embedded in methyl methacrylate resin according to the standard protocols (Iezaki et al., 2016). The bone volume over tissue volume (BV/TV) ratio, which is one of the trabecular bone structural parameters, was measured using Von Kossa staining. The bone formation rate (BFR), which is the calculated rate at which the cancellous bone surface and bone volume are replaced annually as an index of osteoblast activity, was analyzed using the calcein double-labeling method. Calcein was injected into mice twice at an interval of 3 days; 2 days after the last injection, the mice were killed. Osteoclast parameter was analyzed by staining with tartrate-resistant acid phosphatase (TRAP). Analyses were performed using the Osteomeasure Analysis System (Osteometrics, Atlanta, GA, USA), according to the standard protocol (Parfitt et al., 1987).

Data analysis

The results obtained were expressed as the mean $\pm$ standard error, and the statistical significance was determined by the two-way analysis of variance with Bonferroni/Dunnett post hoc test.

Results and discussion

Oral intake of diet containing *S. cerevisiae* S631 does not change diet intake, water intake and body weight

We administered a diet containing 5% *S. cerevisiae* S631 with high concentration of polyamines (data not shown) orally to ovariectomized mice for 28 consecutive days. No considerable change was observed in the daily diet intake (Fig. 1A), daily drinking water intake (Fig. 1B), and body weight (Fig. 1C), irrespective of the diet (normal chow or a diet containing 5% *S. cerevisiae* S631 with high concentration of polyamines) or surgery (sham or OVX) at any time of examination. OVX caused drastic decrease in the uterine weight of mice fed with normal chow at 28 days after the operation; however, the daily oral intake of diet containing 5% *S. cerevisiae* S631 with high concentration of polyamines did not considerably affect the OVX-induced loss of uterine weight (Fig. 1D).

Fig. 2 Oral supplementation of diet containing high-polyamine yeast inhibits OVX-induced bone loss Eight-week-old female ddY mice were subjected to OVX, followed by daily oral supplementation of a diet containing 5% *S. cerevisiae* S631 with high concentration of polyamines for 28 consecutive days ($n = 10$). Typical pictures of Von

Kossa staining are shown in the panel (A), while quantitative BV/TV data are shown in the panel (B). Bar = $200 \mu\text{m}$. ** $p < 0.01$, significantly different from control value obtained in sham-operated mice. # $p < 0.05$, significantly different from the value obtained in mice fed normal chow

Oral intake of diet containing *S. cerevisiae* S631 inhibits OVX-induced bone loss

Under the abovementioned experimental conditions, a marked reduction was noted in the cancellous bone stained by Von Kossa staining, which detects calcium deposits in the tissue, in the vertebrae of ovariectomized mice compared with those in sham-operated mice under normal chow-feeding conditions but not under a diet containing 5% *S. cerevisiae* S631-feeding conditions (Fig. 2). However, we found that supplementation with diet containing 5% *S. cerevisiae* S631 having high concentration of polyamines considerably repressed the reduction of the BV/TV ratio in the cancellous bone of ovariectomized mice, as seen in ovariectomized mice treated with β E2 [BV/TV (%), 26.5 ± 1.0 (n = 5)]; however, no change was observed in sham-operated mice (Fig. 2).

Oral intake of diet containing *S. cerevisiae* S631 inhibits OVX-induced osteoclastic activation

Further, we determined the cellular basis of the preventive effect of diet containing 5% *S. cerevisiae* S631 with high concentration of polyamines on bone loss by histomorphometric analyses of the vertebrae. A considerable increase was noted in the osteoclastic index, extent of the osteoclast surface/bone surface (Oc.S/BS), in the vertebrae of ovariectomized mice fed with normal chow but not with a diet containing 5% *S. cerevisiae* S631 (Fig. 3A, B). The administration of a diet containing 5% *S. cerevisiae* S631 with high concentration of polyamines resulted in a considerable inhibition of the increase in Oc.S/BS to a level observed in sham-operated mice as observed in ovariectomized mice treated with β E2 [Oc.S/BS (%), 8.7 ± 1.3 (n = 5)] (Fig. 3A, B). Contrarily, BFR (the osteoblastic index) was not considerably increased in the vertebrae of

Fig. 3 Oral supplementation of diet containing high-polyamine yeast inhibits OVX-induced osteoclastic activation and increases bone formation in sham-operated mice. Eight-week-old female ddY mice were subjected to OVX, followed by daily oral supplementation of a diet containing 5% *S. cerevisiae* S631 with high concentration of polyamines, and subsequent determination of parameters for bone resorption and bone formation on 28 days after operation (n = 10). Typical pictures of TRAP staining are shown in the panel (A), while

quantitative Oc.S/BS data are shown in the panel (B). Typical pictures of calcein labelling are shown in the panel (C), while quantitative BFR data are shown in the panel (D). ** $p < 0.01$, significantly different from the value obtained in sham-operated mice. # $p < 0.05$, # $p < 0.01$, significantly different from control value obtained in mice fed normal chow. Double-headed white arrows indicate the distance between calcein double labelling (C). Bar = 100 μm (A) and 10 μm (C).

ovariectomized mice, irrespective of their diet (Fig. 3C, D). However, the administration of the diet containing 5% *S. cerevisiae* S631 with high concentration of polyamines considerably increased BFR in the vertebrae of sham-operated mice, but not in that of ovariectomized mice (Fig. 3C, D).

The importance of the results of the current study is that daily oral supplementation of diet containing *S. cerevisiae* S631 with high concentration of polyamines could considerably prevent osteoclastic activation in ovariectomized mice, which would result in the inhibition of bone loss in vivo. Previously, we have demonstrated that daily oral administration of polyamines (spermidine and spermine) at a concentration of 0.3 mM prevented osteoclast activation and bone loss in ovariectomized mice (Yamamoto et al., 2012), which indicates that OVX-induced osteoclast activation and bone loss can be prevented in mice receiving spermidine (0.17 mg/day/mouse) and spermine (0.24 mg/day/mouse) daily via drinking water. Considering the average daily intake of diet containing high-polyamine yeast (approximately 3.4 g/day/mouse) (Fig. 1A), the mice were administered with spermidine (0.35 mg/day/mouse) and spermine (0.037 mg/day/mouse) daily for 28 consecutive days under this experimental protocol. Accordingly, the dose of diet containing 5% *S. cerevisiae* S631 with high concentration of polyamines used in this study appears to be appropriate from the perspective of the pharmacological relevance to the daily administration of spermidine rather than that of spermine.

Notably, diet containing *S. cerevisiae* S631 with high concentration of polyamines could considerably increase BFR in sham-operated mice. Moreover, a trend of increase in BFR was noted in ovariectomized mice fed with a diet containing *S. cerevisiae* S631 with high concentration of polyamines. Accordingly, a diet containing high-polyamine yeast may prevent bone loss through activation of osteoblastic function along with its inhibitory effect on the osteoclastic function in ovariectomized mice. However, the fact that polyamines (spermidine and spermine) did not affect the osteoblastic parameter in OVX mice (Yamamoto et al., 2012) suggests the need to consider the possibility of increase in BFR by components other than polyamines (spermidine and spermine) in diet containing high-polyamine yeast.

In conclusion, based on the study outcomes, we propose that *S. cerevisiae* S631, which contains higher concentration of polyamines than that in commercially available *S. cerevisiae*, could be beneficial for maintaining bone health and the prophylaxis of menopausal osteoporosis against

osteoclast differentiation and maturation. We should perform further investigation, including clinical studies, to apply our findings to human beings.

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Compliance with ethical standards

Conflict of interest Junichi Node and Shigeru Hiramoto are employees of Nisshin Pharma Inc. All other authors state that they have no conflicts of interest.

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