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Dietary Supplementation with Isoflavones Prevents Muscle Wasting in Tumor-Bearing Mice

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Summary Proinflammatory cytokines contribute to the progression of muscle wasting caused by ubiquitin-proteasome-dependent proteolysis. We have previously demonstrated that isoflavones, such as genistein and daidzein, prevent TNF-α-induced muscle atrophy in C2C12 myotubes. In this study, we examined the effect of dietary flavonoids on the wasting of muscle. Mice were divided into the following four groups: vehicle-injected (control) mice fed the normal diet (CN); tumor-bearing mice fed the normal diet (TN); control mice fed the isoflavone diet (CI); and tumor-bearing mice fed the isoflavone diet (TI). There were no significant differences in the intake of food or body weight gain among these four groups. The wet weight and myofiber size of gastrocnemius muscle in TN significantly decreased, compared with those in CN. Interestingly, the wet weight and myofiber size of gastrocnemius muscle in TI were nearly the same as those in CN and CI, although isoflavone supplementation did not affect the increased tumor mass or concentrations of proinflammatory cytokines, such as TNF-α and IL-6, in the blood. Moreover, increased expression of muscle-specific ubiquitin ligase genes encoding MAFbx/Atrogin-1 and MuRF1 in the skeletal muscle of TN was significantly inhibited by the supplementation of isoflavones. In parallel with the expression of muscle-specific ubiquitin ligases, dietary isoflavones significantly suppressed phosphorylation of ERK in tumor-bearing mice. These results suggest that dietary isoflavones improve muscle wasting in tumor-bearing mice via the ERK signaling pathway mediated-suppression of ubiquitin ligases in muscle cells.

Key Words muscle wasting, ubiquitin ligase, isoflavone, tumor

Cachexia is a syndrome that includes wasting of bodily energy reserves and is characterized by loss of muscle with or without loss of fat mass (*1*). It has been reported that skeletal muscle in tumor-bearing mice decreases as a consequence of tumor growth (*2*). Muscle in cachectic individuals exhibits increased protein catabolism and decreased protein synthesis, leading to muscle atrophy. The increase in muscle protein degradation through the ubiquitin-proteasome-dependent proteolytic pathway is due to activation of muscle-specific atrophy-related genes encoding MAFbx/Atrogin-1 and MuRF1. Indeed, previous reports have shown that knockout mice lacking MAFbx/Atrogin-1 and MuRF1 are resistant to denervation-induced muscle atrophy (*3*, *4*). In mice bearing colon-26 (C26) adenocarcinoma cells (5), or Lewis lung carcinoma (LLC) cells (*6*), expression of MAFbx/Atrogin-1 and MuRF1 has been shown to be increased in muscle, suggesting a role for ubiquitin-proteasome-dependent proteolysis in cachectic muscle.

It has been reported that increased levels of serum inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6 and interferon (IFN)-γ, were observed in tumor-bearing mice (*7*–*9*). Li et al. have shown that TNF-α-stimulated MAFbx/Atrogin-1 expression is mediated by activation of the protein kinases (MAPKs): p38, ERK1/2 and JNK (*10*). Indeed, the upregulated expressions of MAFbx/Atrogin-1 mRNA and protein levels induced by tumor bearing were significantly suppressed by administration of the p38α/β MAPK inhibitor SB202190 or the ERK inhibitor PD98059, resulting in improvement of muscle wasting (*11*, *12*). Thus, MAPK-signaling pathways play an important role in the ubiquitin-dependent proteolysis of muscle protein occurring as a result of tumor bearing.

Genistein and daidzein, which are abundantly present in soy products, are also isoflavones that have diverse biological functions, including anti-allergic, anti-inflammatory, antioxidant and anti-cancer as well

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as estrogenic action (*13–15*). Our previous study demonstrated that isoflavones prevent TNF-α-induced myotube atrophy in C2C12 myotubes (*16*). In addition, it has been reported that the cytoprotective effects of genistein as an anti-inflammatory agent are also mediated through the suppression of ERK signaling in pancreatic β-cells and skin (*15*, *17*). However, effects of isoflavones such as genistein and daidzein in counteracting muscle atrophy caused by cancer in vivo are still unknown. In the present study, we examined the effect of isoflavones derived from soy extracts on the muscle of tumor-bearing mice. Mice fed with isoflavones showed inhibition of tumor-associated muscle wasting, and this effect was mediated through the inhibition of ERK signaling, resulting in decreased muscle-specific ubiquitin ligase expression. Our results suggest that supplementation with isoflavones might be beneficial in preventing muscle wasting caused by cancer cachexia.

MATERIALS AND METHODS

Cell culture. LLC cells were purchased from DS Pharma Biomedical Co. (Osaka, Japan) and cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and penicillin-streptomycin mixed solution (Nacalai Tesque, Inc., Kyoto, Japan) at 37°C with 5% CO_2.

Animal model (tumor-bearing). Male C57BL/6 mice (Kyudo, Kumamoto, Japan) at 7 wk, were housed in a room maintained at 24±1°C on a 12-h light/dark cycle with food (Oriental Yeast Co., Ltd., Tokyo, Japan) and water available ad libitum. After acclimatization for 1 wk, mice were randomized and divided into four groups: Hanks' balanced salt solution (HBSS)-injected (control) mice fed the normal diet (CN); tumor-bearing mice fed the normal diet (TN); control mice fed the isoflavone diet (CI); and tumor-bearing mice fed the isoflavone diet (TI). LLC (1×10^6) cells were injected subcutaneously into the right thighs of C57BL/6 mice at 8 wk of age. Mice continued to receive the normal or isoflavone diet until the termination of the experiment 3 wk later. The α-starch content of the isoflavone diet was reduced to adjust for the composition of other nutrients, and comprised a normal diet (based on AIN-93M) mixed with soya flavone HG (0.4% w/w; Fuji Oil Co., Osaka, Japan). The content of isoflavones in soya flavone HG was 40.74% and the specific components have been described in a previous report (*18*). The right hindlimb skeletal muscles, such as the tibialis anterior (TA), extensor digitorum longus (EDL), gastrocnemius (GA), and soleus (So) muscles, were isolated at the time of sacrifice. After measuring the wet weight, the skeletal muscles were immediately frozen in chilled isopentane and liquid nitrogen and were stored at −80°C until analysis. All animal experiments involving tumor-bearing mice were approved by the Committee on Animal Experiments of Nagasaki University, and were performed according to the guidelines for the care and use of laboratory animals set by the University (Nagasaki, Japan).

Quantitative RT-PCR. Total RNA was extracted from mouse GA muscle with an acid guanidinium thiocyanate-phenol-chloroform mixture (ISOGEN™; Nippon Gene, Tokyo, Japan; Invitrogen). Quantitative RT-PCR was performed with appropriate primers and SYBR Green dye using a real-time PCR system (ABI Real-Time PCR Detection System; Applied Biosystems, Foster City, CA), as described previously (*19*). The following primers were used for amplification: 5′-GGCGGACGGCTGGAA-3′ and 5′-CAGATTCTCCTTACTGTATACCTCCTTGT-3′ for mouse MAFbx/Atrogin-1; 5′-TGCCTGGAGATGTTTAC-CAAGC-3′ and 5′-AAACGACCTCCAGACATGGACA-3′ for mouse MuRF1; and 5′-ACCCAGAAGACTGTGGA-TGG-3′ and 5′-TCAGCTCTGGGATGACCTT-3′ for mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Immunoblotting. Mouse GA muscle was prepared in 50 mM Tris-HCl buffer, pH 7.5, containing 150 mM NaCl, 1% Triton X-100, 10 mM NaF, 2 mM Na_3VO_4, and a protease inhibitor cocktail with EDTA (Roche Diagnostics, Tokyo, Japan), and samples were homogenized using a sonicator. The Pierce BCA assay (Pierce, Rockford, IL) was used to quantify proteins. Protein samples were combined with 4×sample buffer (250 mM Tris-HCl, 8% SDS, 40% glycerol, 8% beta-mercaptoethanol, 0.02% bromophenol blue) and separated on a polyacrylamide gel. The proteins were transferred to a polyvinylidene difluoride membrane and probed with primary antibody according to the manufacturer's instructions; anti-phosphorylated ERK1/2, anti-ERK1/2, anti-phosphorylated p38, anti-p38 (Cell Signaling Technology, Danvers, MA), and anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA) antibodies were used. Secondary antibodies were donkey anti-rabbit 1 : 5,000 (GE Healthcare, Little Chalfont, UK). Membranes were developed using Amersham™ ECL™ western blotting detection reagents (GE Healthcare).

Histological analysis and measurement of cross-sectional area. The isolated GA muscle of mice was immediately frozen in chilled isopentane and liquid nitrogen and stored at −80°C until analysis. Sections of GA (5 μm) were fixed in ice-cold acetone. The sections were stained with hematoxylin and eosin (H.E.). Images were acquired with a BIOREVO BZ-9000 microscope (Keyence, Osaka, Japan) using a camera and processed using BZ-II analysis software. To calculate the cross-sectional area (CSA) of GA muscle, at least 200 myofibers per mouse were examined. Data are expressed as fiber size distribution.

Measurement of cytokines. The mice were anesthetized and blood samples were collected from their hearts. Serum was obtained by centrifugation at 1,800 ×g for 20 min at 4°C, and was maintained at −80°C until analysis. Concentrations of IL-6 and TNF-α were determined by using ELISA kits (Pierce; Ray Biotech, Norcross, GA) according to the manufacturer's instructions.

Statistical analysis. All data were statistically evaluated by analysis of variance with Excel-Toukei version 6.0 software (Statistics Survey System-development, Tokyo, Japan) and are expressed as mean±SD, n=5–6. Individual differences between groups were assessed using Scheffe's multiple range test. Differences were considered significant at $p<0.05$.

RESULTS

Effect of dietary isoflavones on muscle mass and myofiber size distribution in tumor-bearing and control mice

To examine the effect of isoflavones, such as genistein and daidzein, on weight gain in mice, we compared food intake and body weight gain in vehicle-injected and tumor-bearing mice fed the normal or isoflavone diet for 3 wk. The food intake in the different groups was CN, 2.5±0.5; TN, 2.7±0.3; CI, 2.8±0.1; and TI, 2.7±0.1 g/d over the 3-wk period. Consistent with this finding, there were no significant differences in weight gain between the normal and soy isoflavone diet groups (Fig. 1). On the other hand, net body weights in TN and TI were slightly lower than those in CN and CI, whereas there were no significant differences in net body weight between TN and TI (Table 1).

Next, we examined the effect of isoflavones on tumor-induced muscle wasting. The wet weights of TA, EDL, GA and So muscles in the TN group were smaller than those in the CN group (Table 1). In particular, the wet weight of the GA in the TN group was significantly decreased compared with the CN. In contrast, the wet weight of GA in the TI group was significantly higher than that in the TN group. The wet weight of skeletal muscle in TI was nearly the same as that in CN and CI (Table 1). Interestingly, the isoflavone diet had no effect on tumor mass (Table 1), even though mice fed the isoflavone diet showed significant resistance to muscle atrophy caused by tumor bearing (Table 1). Similarly, the concentration of blood IL-6 in TI was same as that in TN, whereas the concentrations of IL-6 were increased by tumor-bearing in comparison to control mice (Fig. 2A). In contrast, the blood concentration of TNF-α was hardly affected by tumor implantation (Fig. 2B). The supplementation of isoflavones did not change the concentrations of IL-6 or TNF-α in blood (Fig. 2). These findings suggest that isoflavones specifically act on the muscle, but not the tumor, to prevent muscle atrophy.

To elucidate the effect of isoflavones on tumor-induced myofiber atrophy, we performed histological analysis of the GA muscle in the CN, TN, CI and TI

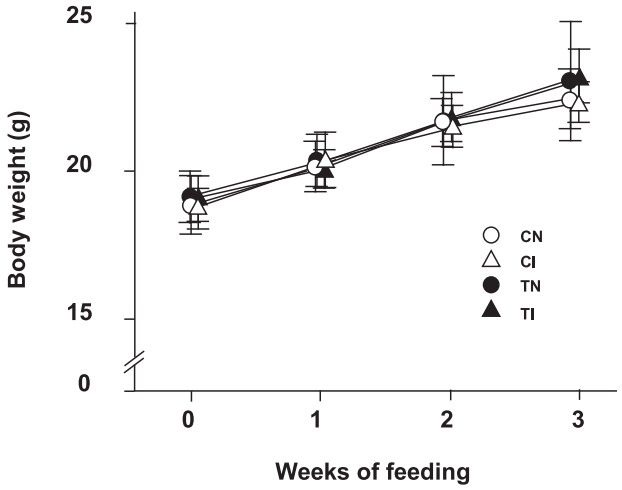

Fig. 1. Changes in body weight with the isoflavone diet. An isoflavone diet or normal diet was given to mice for 3 wk after injection of Hanks' balanced salt solution (HBSS) or LLC cells (CN, n=6; CI, n=6; TN, n=5; TI, n=6). CN, HBSS injected (control) mice fed the normal diet; CI, control mice fed the isoflavone diet; TN, tumor-bearing mice fed the normal diet; TI, tumor-bearing mice fed the isoflavone diet.

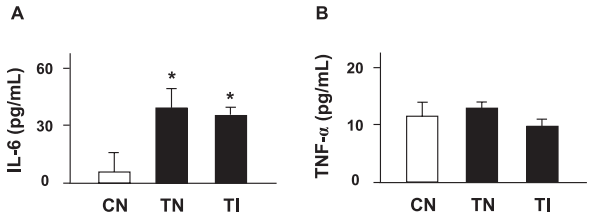

Fig. 2. Changes in blood IL-6 and TNF-α concentrations with the isoflavone diet. Measurements of serum IL-6 (A) and TNF-α (B) levels in CN, TN, TI were performed by ELISA. Data are mean±SD (n=5–6). *p<0.05, compared with HBSS-injected (control) mice fed the normal diet (CN). TN, tumor-bearing mice fed the normal diet; TI, tumor-bearing mice fed the isoflavone diet.

Table 1. Changes in body weight and muscle mass of tumor-bearing mice fed a normal or isoflavone diet.

Groups	CN	TN	CI	TI
n	6	5	6	6
BW (g)	22.4±1.0	23.0±2.0	22.3±0.7	23.2±0.9
Tumor (g)	—	1.40±0.53	—	1.57±0.29
Net BW (g)	22.4±1.0	21.6±1.7	22.3±0.7	21.7±1.1
TA (mg)	41.7±1.0	36.6±4.1	41.9±3.7	39.2±2.1
EDL (mg)	9.8±1.8	9.1±1.4	9.7±0.6	9.9±1.3
GA (mg)	142.5±2.7	115.7±17.3*	140.8±8.6	134.7±7.5†
So (mg)	8.1±0.7	7.0±1.3	8.8±0.9	8.1±1.2

*p<0.05, compared with vehicle-injected (control) mice fed the normal diet (CN). †p<0.05, compared with tumor-bearing mice fed the normal diet (TN).

CI, control mice fed the isoflavone diet; TI, tumor-bearing mice fed the isoflavone diet; BW, body weight; TA, tibialis anterior muscle; EDL, extensor digitorum longus muscle; GA, gastrocnemius muscle; So, soleus muscle.

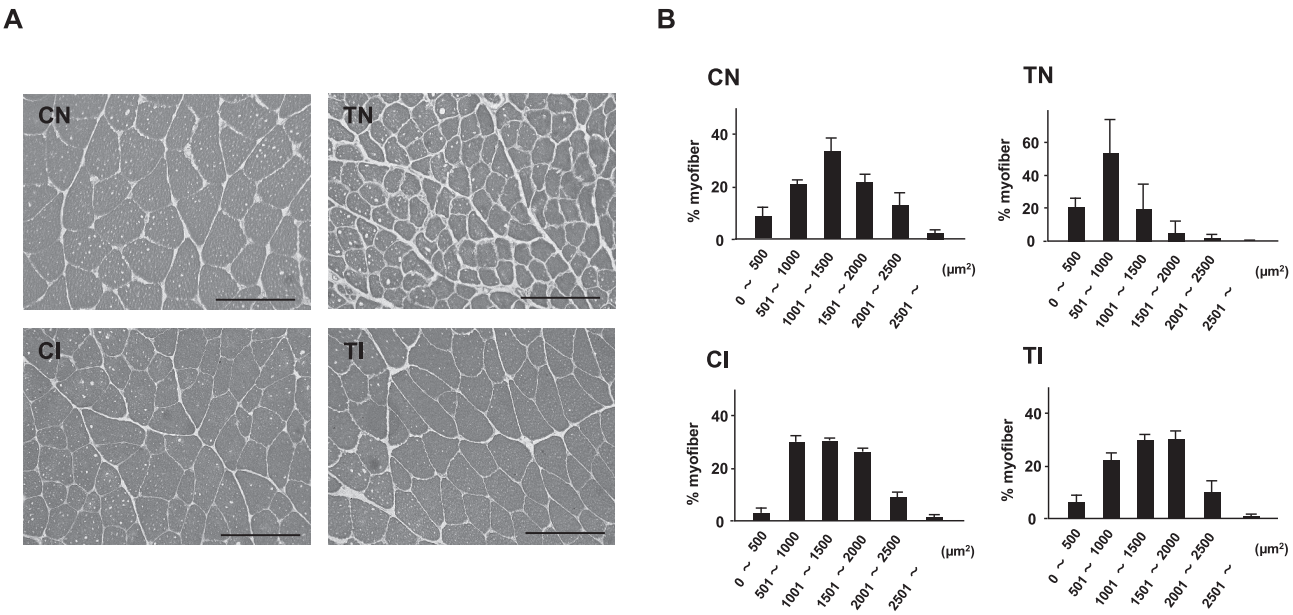

Fig. 3. Effect of dietary isoflavones on tumor-induced decrease in muscle cross-sectional area. Mice were fed the isoflavone diet or normal diet for 3 wk after injection of HBSS or LLC cells. (A) Sections (5-μm thickness) of gastrocnemius (GA) muscle from CN, TN, CI and TI groups were stained with hematoxylin and eosin (H.E.). Scale bar=100 μm. (B) Frequency histograms showing the distribution of cross-sectional areas (μm^2) were calculated. Data are mean±SD (n=5–6). CN, HBSS-injected mice fed the normal diet; TN, tumor-bearing mice fed the normal diet; CI, HBSS-injected mice fed the isoflavone diet; TI, tumor-bearing mice fed the isoflavone diet.

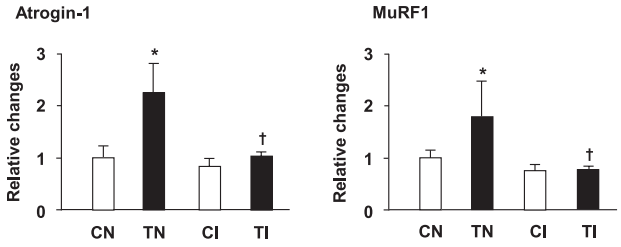

Fig. 4. Effect of dietary isoflavones on ubiquitin ligases, MAFbx/Atrogin-1 and MuRF1 expression in muscles of tumor-bearing mice. Total RNA of gastrocnemius muscle was extracted and subjected to real-time RT-PCR. The intensity ratio of MAFbx/Atrogin-1 or MuRF1 to GAPDH was calculated. Data are mean±SD (n=5–6). $^{*}p<0.05$, compared with HBSS-injected (control) mice fed the normal diet (CN). $^{\dagger}p<0.05$, compared with tumor-bearing mice fed the normal diet (TN). CI, HBSS-injected mice fed the isoflavone diet; TI, tumor-bearing mice fed the isoflavone diet.

groups. The CSA of myofibers stained with H.E. in the TN group, was smaller than in the CN group (Fig. 3A). In contrast, the CSA of myofibers in the TI group was similar to that observed in the CN and CI groups (Fig. 3A). In comparison to the size distribution of myofibers in the CN group, a leftward shift in the size distribution of myofibers was observed in the TN group (Fig. 3B). The size distributions of myofibers in the TI groups were nearly the same as those in the CN and CI groups (Fig. 3B). In particular, no leftward shift in the size distributions of myofibers caused by tumor implantation was observed in the TI groups.

Effect of dietary isoflavones on expression of atrophy-related genes in tumor bearing

Muscle atrophy-associated ubiquitin ligases, such as MAFbx/Atrogin-1 and MuRF1, contribute to skeletal muscle atrophy (*3*, *4*). It has been shown that tumor implantation is associated with an increase in the expression of the ubiquitin ligases MAFbx/Atrogin-1 and MuRF1 (*20*). To investigate whether isoflavones suppress muscle wasting through ubiquitin ligase activation, we examined the expression of MAFbx/Atrogin-1 and MuRF1 mRNA levels in TN, CN, CI and TI mice. The expression of MAFbx/Atrogin-1 and MuRF1 mRNA in the skeletal muscle of tumor-bearing mice was significantly increased, compared with that of vehicle-injected mice (Fig. 4). This increased expression of MAFbx/Atrogin-1 and MuRF1 mRNA in skeletal muscle of tumor-bearing mice was significantly inhibited by dietary isoflavones (Fig. 4).

Effect of dietary isoflavones on MAPK signaling in tumor-bearing muscle

ERK and p38 signaling is a well-known MAPK pathway mediating oxidative stress or cytokine production (*10*). Therefore, we investigated the effect of isoflavones on phosphorylation of ERK and p38 in tumor-bearing muscle. The phosphorylation of ERK in the skeletal muscle of TN mice was significantly increased, compared with that of CN mice, whereas the phosphorylation of p38 was not influenced by the presence of tumors (Fig. 5). Dietary isoflavones significantly suppressed phosphorylation of ERK in TI mice, corresponding to the upregulation of atrogenes induced by tumor bearing (Fig. 5).

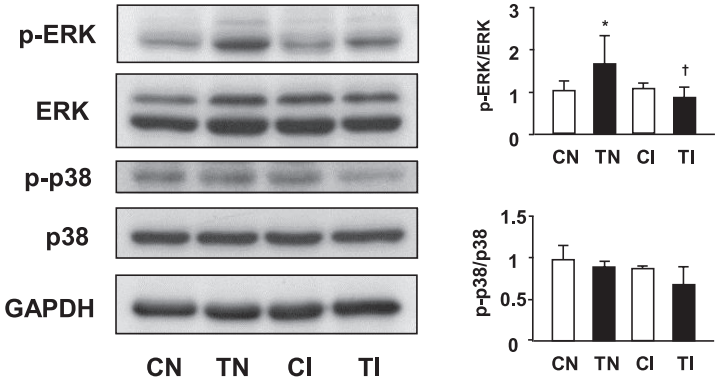

Fig. 5. Effect of isoflavones on phosphorylation of ERK and p38 in muscles of tumor-bearing mice. Proteins (20 μg/lane) extracted from gastrocnemius muscle were subjected to SDS-PAGE and transferred to a polyvinylidene difluoride membrane. Immunoblotting for phosphorylated-ERK and p38 were performed on different membranes without stripping, as described in "Materials and Methods." Densitometric ratios of phosphorylated-ERK to ERK or phosphorylated-p38 to p38 were calculated. Data are mean±SD (n=5–6). $^{*}p<0.05$, compared with HBSS-injected (control) mice fed the normal diet (CN). $^{\dagger}p<0.05$, compared with tumor-bearing mice fed the normal diet (TN). CI, HBSS-injected mice fed the isoflavone diet; TI, tumor-bearing mice fed the isoflavone diet.

DISCUSSION

The hyperactivation of ERK in skeletal muscle has been reported to contribute to the onset of muscle wasting (*2*, *12*). In fact, administration of the ERK inhibitor PD98059 attenuates the expression of MAFbx/Atrogin-1 mRNA induced by tumor bearing, resulting in improvement of impaired muscle function (*12*). Thus, the phosphorylation of ERK plays an important role in the development of muscle wasting. Consistent with previous reports, our results also showed that the phosphorylation of ERK in skeletal muscle was enhanced by the transplantation of tumors (Fig. 5). Furthermore, the intake of dietary isoflavones containing genistein and daidzein suppressed the activation of ERK in skeletal muscle caused by tumor-bearing. In a previous study, quercetin, a natural flavonoid, was shown to significantly diminish the activation of ERK-, p38 MAPK- and NFκB-signaling molecules, resulting in protection against obesity-induced skeletal muscle atrophy (*21*). In addition, our previous data showed that antioxidative nutrients, such as catechins and quercetin, suppress MAFbx/Atrogin-1 and MuRF1 expression in skeletal muscle cells, possibly through inhibition of ERK signaling (*22*). These findings indicate that improvement of disturbances in ERK signaling prevents muscle atrophy.

It has been reported that treatment of LLC cell-conditioned medium in C2C12 myotubes resulted in hyperproduction of reactive oxygen species (ROS) through a decrease of the oxygen consumption rate (*23*). Moreover, Yu et al. showed that cachectic stimuli, such as LPS and TNF-α, lead to increases in oxidative stress and consequent activation of protein degradation pathway related-upregulation of MAFbx/Atrogin-1 and MuRF1 mRNAs in skeletal muscle (*24*). Thus, ROS produced as a result of oxidative stress contribute to development of muscle wasting, which occurs as a result of increased levels of muscle-specific ubiquitin ligases. A recent report showed that deficiency of the smad3 gene, which is a downstream signaling molecule for TGF-β and myostatin, resulted in myostatin-induced ROS induction through the p38 and ERK MAPK pathways in skeletal muscle, leading to muscle atrophy (*25*). Given the importance of ROS in the development and cause of muscle proteolysis, inhibition of the MAPK-ROS signaling pathway by antioxidants and related nutrients may be critical for the development of new therapeutic approaches.

Isoflavones have been shown to exhibit an antioxidant effect (*26–28*). This biological effect of isoflavones occurs by indirect rather than direct action in mammalian cells. In fact, mice fed a methionine-choline-deficient diet, which is associated with development of nonalcoholic steatohepatitis, exhibit increased levels of TBARS and heme oxygenase-1 mRNA, which are significantly mitigated by genistein supplementation, resulting in prevention of liver inflammation and fibrosis (*29*). Furthermore, genistein inhibited H_2O_2-induced activation of the JNK and ERK signaling pathway, leading to protection of cortical neurons against oxidative stress (*30*). Davis et al. have reported that isoflavone supplementation inhibited TNF-α-induced NFκB DNA binding activity and increased 5-OHmdU levels, an indicator of oxygen free radical-induced DNA damage (*31*). Consistent with these findings, our previous study showed that promoter activity of the ubiquitin ligase MuRF1 was mediated by acetylation of p65, a subunit of NFκB, which is a downstream target of the TNF-α-signaling pathway. This increased MuRF1 promoter activity was abolished by isoflavone treatment, which was associated with deacetylation of p65 (*16*). Therefore, these findings suggested that the biological activity of isoflavones is due to their effect in modulating oxidative stress related-signaling pathways.

It has been reported that epigallocatechin-3-gallate, which is a flavonoid known to be contained in foods including green tea, attenuated skeletal muscle atrophy caused by cancer cachexia due primarily to inhibition

of tumor cell growth (*6*). In contrast, sorafenib, a multikinase inhibitor with anti-tumor activity, exerts its anticachectic action directly on the muscle, but not on the tumor (*2*). We found that isoflavone supplementation improved the loss of muscle weight caused by tumor bearing, but did not affect tumor mass (Table 1) or the concentration of blood IL-6 (Fig. 2). These findings suggest that isoflavones dominantly act on muscle cells. The glycoprotein gp130 is a common cytokine receptor, which is highly expressed in skeletal muscle, and muscle-specific gp130-deficient mice were found to be resistant to muscle loss caused by LLC-induced cachexia (*32*). The phosphorylation of gp130 was induced by IL-6 binding, resulting in activation of its downstream signaling SHP/ERK MAPK cascade (*33*). Therefore, isoflavones, at least in part, may act on gp130-related molecules, thereby preventing muscle wasting through inhibition of the ERK signaling pathway.

It has been reported that increased levels of serum TNF-α and IL-6 were observed in tumor-bearing mice (*7*, *8*). In this study, the concentrations of IL-6 were increased by tumor-bearing in comparison to control mice, whereas the concentrations of TNF-α were hardly affected by tumor implantation (Fig. 2). Das and Hoefler have reported that these discrepancies may be caused by short half-life of TNF-α and variations in sensitivities of the detection methods (*34*). Further investigations are necessary to elucidate the stability of circulating TNF-α in cachexia.

In this study, we report an inhibitory effect of isoflavones on the muscle wasting induced by tumor bearing. Our data also suggest that natural isoflavones derived from soy beans provide a therapeutic benefit by inhibiting enhanced ERK signaling to prevent cancer-related muscle atrophy.

Acknowledgments

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REFERENCES

1) Gordon JN, Green SR, Goggin PM. 2005. Cancer cachexia. *QJM* **98**: 779–788.
2) Toledo M, Penna F, Busquets S, López-Soriano FJ, Argilés JM. 2014. Distinct behaviour of sorafenib in experimental cachexia-inducing tumours: the role of STAT3. *PLoS One* **9**: e113931.
3) Bodine SC, Latres E, Baumhueter S, Lai VK, Nunez L, Clarke BA, Poueymirou WT, Panaro FJ, Na E, Dharmarajan K, Pan ZQ, Valenzuela DM, DeChiara TM, Stitt TN, Yancopoulos GD, Glass DJ. 2001. Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science* **294**: 1704–1708.
4) Gomes MD, Lecker SH, Jagoe RT, Navon A, Goldberg AL. 2001. Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. *Proc Natl Acad Sci USA* **98**: 14440–14445.
5) Asp ML, Tian M, Wendel AA, Belury MA. 2010. Evidence for the contribution of insulin resistance to the development of cachexia in tumor-bearing mice. *Int J Cancer* **126**: 756–763.
6) Wang H, Lai YJ, Chan YL, Li TL, Wu CJ. 2011. Epigallocatechin-3-gallate effectively attenuates skeletal muscle atrophy caused by cancer cachexia. *Cancer Lett* **305**: 40–49.
7) Costelli P, Carbó N, Tessitore L, Bagby GJ, Lopez-Soriano FJ, Argilés JM, Baccino FM. 1993. Tumor necrosis factor-alpha mediates changes in tissue protein turnover in a rat cancer cachexia model. *J Clin Invest* **92**: 2783–2789.
8) Strassmann G, Fong M, Kenney JS, Jacob CO. 1992. Evidence for the involvement of interleukin 6 in experimental cancer cachexia. *J Clin Invest* **89**: 1681–1684.
9) Matthys P, Heremans H, Opdenakker G, Billiau A. 1991. Anti-interferon-gamma antibody treatment, growth of Lewis lung tumours in mice and tumour-associated cachexia. *Eur J Cancer* **27**: 182–187.
10) Li YP, Chen Y, John J, Moylan J, Jin B, Mann DL, Reid MB. 2005. TNF-alpha acts via p38 MAPK to stimulate expression of the ubiquitin ligase atrogin1/MAFbx in skeletal muscle. *FASEB J* **19**: 362–370.
11) Zhang G, Jin B, Li YP. 2011. C/EBPβ mediates tumour-induced ubiquitin ligase atrogin1/MAFbx upregulation and muscle wasting. *EMBO J* **30**: 4323–4335.
12) Penna F, Costamagna D, Fanzani A, Bonelli G, Baccino FM, Costelli P. 2010. Muscle wasting and impaired myogenesis in tumor bearing mice are prevented by ERK inhibition. *PLoS One* **5**: e13604.
13) Mahmoud AM, Yang W, Bosland MC. 2014. Soy isoflavones and prostate cancer: a review of molecular mechanisms. *J Steroid Biochem Mol Biol* **140**: 116–132.
14) Yamashita S, Tsukamoto S, Kumazoe M, Kim YH, Yamada K, Tachibana H. 2012. Isoflavones suppress the expression of the FcεRI high-affinity immunoglobulin E receptor independent of the estrogen receptor. *J Agric Food Chem* **60**: 8379–8385.
15) Lee TH, Do MH, Oh YL, Cho DW, Kim SH, Kim SY. 2014. Dietary fermented soybean suppresses UVB-induced skin inflammation in hairless mice via regulation of the MAPK signaling pathway. *J Agric Food Chem* **62**: 8962–8972.
16) Hirasaka K, Maeda T, Ikeda C, Haruna M, Kohno S, Abe T, Ochi A, Mukai R, Oarada M, Eshima-Kondo S, Ohno A, Okumura Y, Terao J, Nikawa T. 2013. Isoflavones derived from soy beans prevent MuRF1-mediated muscle atrophy in C2C12 myotubes through SIRT1 activation. *J Nutr Sci Vitaminol* **59**: 317–324.
17) Kim EK, Kwon KB, Song MY, Seo SW, Park SJ, Ka SO, Na L, Kim KA, Ryu DG, So HS, Park R, Park JW, Park BH. 2007. Genistein protects pancreatic beta cells against cytokine-mediated toxicity. *Mol Cell Endocrinol* **278**: 18–28.
18) Kai M, Yamauchi A, Tominaga K, Koga A, Kai H, Kataoka Y. 2004. Soybean isoflavones eliminate nifedipine-induced flushing of tail skin in ovariectomized mice. *J Pharmacol Sci* **95**: 476–478.
19) Nakao R, Hirasaka K, Goto J, Ishidoh K, Yamada C, Ohno A, Okumura Y, Nonaka I, Yasutomo K, Baldwin KM, Kominami E, Higashibata A, Nagano K, Tanaka

K, Yasui N, Mills EM, Takeda S, Nikawa T. 2009. Ubiquitin ligase Cbl-b is a negative regulator for insulin-like growth factor 1 signaling during muscle atrophy caused by unloading. *Mol Cell Biol* **29**: 4798–4811.

20) Chen JA, Splenser A, Guillory B, Luo J, Mendiratta M, Belinova B, Halder T, Zhang G, Li YP, Garcia JM. 2015. Ghrelin prevents tumour- and cisplatin-induced muscle wasting: characterization of multiple mechanisms involved. *J Cachexia Sarcopenia Muscle* **6**: 132–143.

21) Le NH, Kim CS, Park T, Park JH, Sung MK, Lee DG, Hong SM, Choe SY, Goto T, Kawada T, Yu R. 2014. Quercetin protects against obesity-induced skeletal muscle inflammation and atrophy. *Mediators Inflamm* **2014**: 834294.

22) Hemdan DI, Hirasaka K, Nakao R, Kohno S, Kagawa S, Abe T, Harada-Sukeno A, Okumura Y, Nakaya Y, Terao J, Nikawa T. 2009. Polyphenols prevent clinorotation-induced expression of atrogenes in mouse C2C12 skeletal myotubes. *J Med Invest* **56**: 26–32.

23) McLean JB, Moylan JS, Andrade FH. 2014. Mitochondria dysfunction in lung cancer-induced muscle wasting in C2C12 myotubes. *Front Physiol* **5**: 503.

24) Yu Z, Li P, Zhang M, Hannink M, Stamler JS, Yan Z. 2008. Fiber type-specific nitric oxide protects oxidative myofibers against cachectic stimuli. *PLoS One* **3**: e2086.

25) Sriram S, Subramanian S, Juvvuna PK, Ge X, Lokireddy S, McFarlane CD, Wahli W, Kambadur R, Sharma M. 2014. Myostatin augments muscle-specific ring finger protein-1 expression through an NF-κB independent mechanism in SMAD3 null muscle. *Mol Endocrinol* **28**: 317–330.

26) Li Y, Kong D, Ahmad A, Bao B, Sarkar FH. 2013. Antioxidant function of isoflavone and 3,3′-diindolylmethane: are they important for cancer prevention and therapy? *Antioxid Redox Signal* **19**: 139–150.

27) Exner M, Hermann M, Hofbauer R, Kapiotis S, Quehenberger P, Speiser W, Held I, Gmeiner BM. 2001. Genistein prevents the glucose autoxidation mediated atherogenic modification of low density lipoprotein. *Free Radic Res* **34**: 101–112.

28) Patel RP, Boersma BJ, Crawford JH, Hogg N, Kirk M, Kalyanaraman B, Parks DA, Barnes S, Darley-Usmar V. 2001. Antioxidant mechanisms of isoflavones in lipid systems: paradoxical effects of peroxyl radical scavenging. *Free Radic Biol Med* **31**: 1570–1581.

29) Yoo NY, Jeon S, Nam Y, Park YJ, Won SB, Kwon YH. 2015. Dietary supplementation of genistein alleviates liver inflammation and fibrosis mediated by a methionine-choline-deficient diet in db/db mice. *J Agric Food Chem* **63**: 4305–4311.

30) Qian Y, Cao L, Guan T, Chen L, Xin H, Li Y, Zheng R, Yu D. 2015. Protection by genistein on cortical neurons against oxidative stress injury via inhibition of NF-kappaB, JNK and ERK signaling pathway. *Pharm Biol* **53**: 1124–1132.

31) Davis JN, Kucuk O, Djuric Z, Sarkar FH. 2001. Soy isoflavone supplementation in healthy men prevents NF-kappa B activation by TNF-alpha in blood lymphocytes. *Free Radic Biol Med* **30**: 1293–1302.

32) Puppa MJ, Gao S, Narsale AA, Carson JA. 2014. Skeletal muscle glycoprotein 130's role in Lewis lung carcinoma-induced cachexia. *FASEB J* **28**: 998–1009.

33) Ernst M, Jenkins BJ. 2004. Acquiring signalling specificity from the cytokine receptor gp130. *Trends Genet* **20**: 23–32.

34) Das SK, Hoefler G. 2013. The role of triglyceride lipases in cancer associated cachexia. *Trends Mol Med* **19**: 292–301.