Induction of Growth Differentiation Factor 15 in Skeletal Muscle of Old Taurine Transporter Knockout Mouse

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It has been identified that skeletal muscle is an endocrine tissue. Since skeletal muscle aging affects not only to muscle strength and function but to systemic aging and lifespan, myokines secreted from skeletal muscle may be crucial factors for intertissue communication during aging. In the present study, we investigated the expression of myokines associated with skeletal muscle aging in taurine transporter knockout (TauTKO) mice, which exhibit the accelerated skeletal muscle aging. Among transforming growth factor (TGF)-beta family genes, only growth and differentiation factor 15 (GDF15) was markedly higher (>3-fold) in skeletal muscle of old TauTKO mice compared with that of either young TauTKO mice or old wild-type mice. Circulating levels of GDF15 were also elevated in old TauTKO mice. An elevation in circulating GDF15 was also observed in very old (30-month-old) wild-type mice, while skeletal GDF15 levels were normal. The treatment of cultured mouse C2C12 myotubular cells with aging-related factors that mediate cellular stresses, such as oxidative stress (hydrogen peroxide) and endoplasmic reticulum stress (tunicamycin and thapsigargin), leads to an increase in GDF15 secretion. In conclusion, GDF15 is a myokine secreted by aging-related stress and may control aging phenotype.

Key words growth differentiation factor 15 (GDF15); sarcopenia; aging cell; skeletal muscle; myokine

Aging-related loss of muscle mass and strength causes physical activities of daily living and decreases the QOL.3) Additionally, it has been demonstrated that skeletal muscle mass and strength are well associated with the mortality rate and aging-related diseases in human.2–4) Moreover, several studies in mammals and fruit flies demonstrated that stress-sensing in skeletal muscle influences systemic aging and lifespan,4) suggesting that non-autonomous communication between aged skeletal muscle and whole body may play a key role in aging process. Skeletal muscle is an endocrine organ that produce cytokines and growth factors, which is called as myokines.5) Several myokines have been identified, and these regulate a variety of physical activity. For instance, interleukin-6 (IL-6) and exercise-induced IL-6 increases in blood glucagon-like peptide-1, which may contribute to blood glucose control.7) Although it is assumable that myokines might be also important in non-autonomous communication during aging, little is known about myokines changed in aging.

Some of transforming growth factor β (TGFβ) family proteins contribute to skeletal muscle physiology. For instance, myostatin is expressed specifically in skeletal muscle, and it controls skeletal muscle mass.8,9) Meanwhile, phosphorylated SMAD3, a downstream of TGFβ family protein, is increased in muscle stem (satellite) cells of old mice,10 suggesting some TGFβ family protein may contribute to aging phenotype in skeletal muscle. Growth differentiation factor 15 (GDF15), which is also known as Macrophage inhibitory cytokine-1 (MIC-1), is also a member of TGFβ family.11) It is produced by many kinds of tumors, and plays an important role in anorexia and body weight loss in cancer-bearing animals.12,13) Circular level of GDF15 is elevated in the patients with a various tumors.14) The anorexic effect of GDF15 is mediated by central nerve system.12,15) Its role in anorexia is mediated by glial cell line-derived neurotrophic factor (GDNF) family receptor-α-like (GFRAL), which is recently identified as GDF15 receptor in brain.16–19) Recently, it has been reported that GDF15 is secreted from skeletal muscle in response to mitochondrial stress.20,21)

Exploring the myokine associated with aging-related diseases may contribute to the extension of healthy lifespan. While TGFβ family may be involved in intertissue communication as myokines during aging, how the secretion of TGFβ family is regulated during skeletal muscle aging has not been clarified. Therefore, in this study, we investigated the secretion of cytokines from skeletal muscle of taurine transporter (TauT) knockout mice which exhibits the premature aging-like phenotype in skeletal muscle.22) Then, we identified GDF15 as a secreted growth factor of aged skeletal muscle. We also investigated the regulation of GDF15 expression in vitro model to clarify whether GDF15 is secreted from skeletal muscle cells in response to aging-related stresses.

METHODS

Animal Care The experimental procedures were approved by the Institutional Animal Care and Use Committee of Hyogo University of Health Sciences. Male taurine transporter knockout (TauTKO) and littermate mice were housed in SPF environment, fed a standard chow (MF, Oriental Yeast, Japan), had access to water ad libitum and maintained on a 12-h light/dark cycle.23) Eighteen- to 22-month-old mice were used as old mice and 3-month-old mice were used as young mice.

Cell Culture C2C12 mouse myoblasts was purchased from DS Pharma Biomedical Co. (Osaka, Japan). C2C12
cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum for maintenance. To induce differentiation to myotube, cells were cultured in DMEM containing 2% fetal bovine serum for 1 week. After differentiation, cells were treated with hydrogen peroxide, tunicamycin and thapsigargin.

**Enzyme-Linked Immunosorbent Assay (ELISA)** As soon as the mice was euthanatized, blood was taken by heart puncture and serum was stored at $-80^\circ$C. Culture medium was collected and centrifuged at 12000×g for 10min to remove detached cells. GDF15 level in serum or culture medium was measured by Mouse/Rat GDF-15 Quantikine ELISA Kit (R&D Systems, MN, U.S.A.). According to manufacturer’s datasheet, this assay kit can recognize natural mouse and rat GDF-15, recombinant mouse mature GDF-15, precursor GDF-15, and GDF-15/BMP-2 heterodimer, but not recombinant mouse GDF-15 (monomer) and other GDF proteins, including mouse recombinant GDF-1, 3, 5, 6, 7, 8 and human recombinant GDF-11.

**Quantitative RT-PCR** Total RNA was isolated from murine tibialis anterior (TA) muscle or C2C12 cells by using Sepazol super (Nacalai Tesque, Kyoto, Japan) and cDNA was generated by using Rever Tra Ace (Toyobo, Osaka, Japan). Quantitative RT-PCR analysis was performed by using Applied Biosystems Step One Plus (Applied Biosystems) with THUNDERBIRD SYBR qPCR Mix (Toyobo, Japan). The primers used are as follows; GDF15 forward: 5'-CTC AGA ACC AAG TCC TGA CCC-3', reverse: 5'-CCG GTT GAC GCG GAG TAG-3'. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward: 5'-GCC GGT GCT GAT TGC GCT-3', reverse: 5'-CCCTT TGG CTC CAC CCT T-3'.

**Statistics** Student’s $t$-test, Tukey–Kramer test or Bonferroni test (for multiple comparisons) were used to determine statistical significance between groups. Differences were considered statistically significant when the calculated $p$ value was less than 0.05.

### RESULTS

**Induction in GDF15 in Aged TauTKO Muscle** We have previously reported that TauTKO mice exhibit shortened lifespan. Since the taurine transporter transports taurine from the outside to the inside of cells, organs of TauTKO mice lose taurine. This mouse taurine deficient model shows reduced skeletal muscle weight and the acceleration of skeletal muscle aging. Therefore, we examined whether the TGFβ family genes of old TauTKO muscle were increased in the transcriptome data set which we previously generated to compare the transcriptome profiles of young and old wild-type (WT) and TauTKO mice. Data was obtained from Microarray analysis (GEO accession No. GSE57373). (B) GDF-15 mRNA levels were validated by the qRT-PCR. $n=3$. Serum GDF15 protein level was measured by ELISA. $n=4–8$. ***$p<0.01$ vs. young TauTKO, ****$p<0.01$ vs. old WT.
and TauTKO mice. Of the profile, only growth and differentiation factor 15 (GDF15) is >3-fold higher in old TauTKO muscle compared with that of either old WT muscle or young TauTKO muscle (Fig. 1A). An induction in GDF15 mRNA in old TauTKO muscle was validated by quantitative PCR (Fig. 1B). Serum levels of GDF15 were also increased in TauTKO mouse, as assessed by ELISA (Fig. 1C).

Additionally, we tested skeletal muscle GDF15 mRNA and serum GDF15 protein levels in very old (30-month-old) WT mice (Fig. 2). Serum GDF15 levels were higher in very old WT mice than in those of young mice (Fig. 2A), while mRNA levels of skeletal muscle decreased (Fig. 2B).

**Secretion of GDF15 from C2C12 Myotube in Response to Aging-Related Stresses** Protein homeostasis in endoplasmic reticulum is significant to aging process. Accumulation of intracellular aggregates composed by misfolded proteins during aging deteriorates cellular function. Consistently, we previously found that muscular taurine depletion activates unfolded protein response (UPR) signal pathway, which may be resulted from impairment of protein homeostasis by taurine loss. Therefore, we tested whether endoplasmic reticulum (ER) stress is associated with an induction in GDF15. The cultured C2C12 myotubes were treated with tunicamycin and thapsigargin, inducers of ER stress, and the expression of GDF15 mRNA was measured. Both treatment of thapsigargin (10nm) and tunicamycin (50ng/mL) for 24h induced GDF15 mRNA expression (Fig. 3A). Furthermore, exposure to ER stress increased in GDF15 secretion into the culture medium (Fig. 3B), indicating that GDF15 expression is also regulated by ER stress.

Oxidative stress is also major cellular stress to induce aging, and it can activate UPR pathway. Therefore, we tested...
the effect of hydrogen peroxide treatment. Hydrogen peroxide treatment (0.5 or 1 mM) for 24 h induced GDF15 mRNA in the C2C12 myotubes (Fig. 3C). Moreover, the amount of GDF15 protein secreted into the culture medium was increased by oxidative stress (Fig. 3D).

DISCUSSION

In the present study, we demonstrate that serum GDF15 level is higher in old TauTKO mice than old WT mice and young TauTKO mice. We have previously reported that TauTKO mice exhibit the premature aging-like phenotype in skeletal muscle, including an induction in cyclin-dependent kinase inhibitor 2A (p16INK4A), which is a biomarker gene of cellular senescence, accumulation of abnormal myofibril and a reduction in mitochondrial Complex I activity compared to kinase inhibitor 2A (p16INK4A), which is a biomarker gene of young TauTKO mice. We have previously reported that level is higher in old TauTKO mice than old WT mice and WT or young TauKO mice. 22) One might assume that skeletal muscle of very old WT mice than in that of young WT mice. Nonetheless, expression of GDF15 in mice results in a decrease in food intake and a body weight loss, 25) it is assumable that induction of serum GDF15 level may cause anorexia in the patients. Consistently, 18-month-old TauTKO mice but not WT mice display loss of body weight compared to 12-month-old mice. 22) Our present results indicate that an induction of GDF15 in aged animals may cause to aging-related anorexia and body weight loss.

We also demonstrate that circulation levels of GDF15 are elevated in very old WT mice although GDF15 mRNA of skeletal muscle is lower than young WT mice. Nonetheless, p16INK4a mRNA is only about 2-fold higher in skeletal muscle of very old WT mice than in that of young WT mice (data not shown), although it is more than 10-fold higher in that of old (18-month-old) TauTKO mice than in that of either young WT or young TauKO mice. 22) One might assume that skeletal muscle of very old WT mice is not sufficiently senescent to produce GDF15 and that the increase in circulating GDF15 in very old mice may be caused by the secretion from tissues other than muscle.

Aging in muscle lead to protein homeostasis collapse, and then accumulation of misfolded proteins, which results in the loss of muscle function. 26,27) Protein homeostasis is impaired in skeletal muscle of TauTKO mice. 22) Therefore, we hypothesized that impairment of protein homeostasis is associated with an induction in GDF15 in skeletal muscle. In the present study, we confirmed that initiation of ER stress promotes the secretion of GDF15 from cultured skeletal muscle cells. These data suggest that the impairment of protein homeostasis may be involved in the increase in GDF15 in skeletal muscle during aging in TauTKO mice. However, GDF15 expression in skeletal muscle of 30-month-old WT mice is reduced, although UPR markers, such as Grp78, are also upregulated during muscle aging in WT mice. 24,28) While some signaling proteins, such as inositol-requiring enzyme 1 (IRE1), protein kinase R-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6), are involved in UPR, the involvement of these UPR-signaling pathways in TauTKO and very old WT muscles has not been fully clarified. Further studies will uncover the molecular mechanisms which involved in the GDF15 induction during muscle aging in TauTKO mice.

Since skeletal muscle constitutes 30–50% of total body mass, an increase in GDF15 in skeletal muscle may contribute to significant elevations in circulating GDF15. Increased circulating GDF15 is associated not only with anorexia but also with several age-related diseases, such as cardiac disease, diabetes and neurodegenerative diseases. 29) The induction of GDF15 may contribute to anorexia, a serious mental problem that leads to an eating disorder observed in many patients with late-stage cancer. Notably, serum GDF15 content correlates with the mortality rate in aging-related diseases in humans. 30–33) Future investigation is needed to determine whether the elevated GDF15 in the aged muscle is a therapeutic target to prevent aging-related anorexia, skeletal muscle loss and a various aging-related chronic diseases.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES

1) Argilés JM, Busquets S, Stemmler B, Lopez-Soriano FJ. Cachexia and sarcopenia: Mechanisms and potential targets for intervention. Curr. Opin. Pharmacol., 22, 100–106 (2015).
2) Anker SD, Ponikowski P, Varney S, Chua TP, Clark AL, Webb-Peploe KM, Harrington D, Kox WJ, Poole-Wilson PA, Coats AJ. Wasting as independent risk factor for mortality in chronic heart failure. Lancet, 349, 1050–1053 (1997).
3) Metter EJ, Talbot LA, Schrager M, Conwit S. Skeletal muscle strength as a predictor of all-cause mortality in healthy men. J. Gerontol. A Biol. Sci. Med. Sci., 57, B359–B365 (2002).
4) Demontis F, Piccirillo R, Goldberg AL, Perrimon N. The influence of skeletal muscle on systemic aging and lifespan. Aging Cell, 12, 943–949 (2013).
5) Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. Physiol. Rev., 88, 1379–1406 (2008).
6) Ostrowski K, Rohde T, Zacho M, Asp S, Pedersen BK. Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. J. Physiol., 508, 949–953 (1998).
7) Ellingsgaard H, Hauselmann I, Schuler B, Habib AM, Baggio LL, Meier DT, Epple L, Bouzakri K, Wueest S, Muller YD, Hansen AM, Reinecke M, Konrad D, Gassmann M, Reimann F, Halban PA, Gromada J, Drucker DJ, Gribble FM, Ehses JA, Donath MY, Meier DT, Eppler E, Bouzakri K, Wueest S, Muller YD, Hansen AM, Reinecke M, Konrad D, Gassmann M, Reimann F, Halban PA, Gromada J, Drucker DJ, Gribble FM, Ehses JA, Donath MY. Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. Nat. Med., 17, 1481–1489 (2011).
8) McPherron AC, Lawler AM, Lee S-J. Regulation of skeletal muscle mass in mice by a new TGF-p superfamily member. Nature, 387, 83–90 (1997).
9) White TA, Lebrasseur NK, Myostatin and sarcopenia: opportunities and challenges—A mini-review. Gerontol.ogy, 60, 289–293 (2014).
Carlson ME, Hsu M, Conboy IM. Imbalance between pSmad3 and pSmad1/5/8 mediates Notch-induced CDK inhibitors in old muscle stem cells. Nature, 454, 528–532 (2008).

Bootcov MR, Bauskin AR, Valenzuela SM, Moore AG, Bansal M, He XY, Zhang HP, Donnellan M, Mahler S, Pryor K, Walsh BJ, Nicholson RC, Fairlie WD, Por SB, Robbins JM, Breit SN. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. Proc. Natl. Acad. Sci. U.S.A., 94, 11514–11519 (1997).

Johnen H, Lin S, Kuffner T, Brown DA, Tsai VW, Bauskin AR, Wu L, Pankhurst G, Jiang L, Junankar S, Hunter M, Fairlie WD, Lee NJ, Enriquez RF, Baldock PA, Corey E, Apple FS, Murakami MM, Lin EJ, Wang C, During MJ, Sainsbury A, Herzog H, Breit SN. Tumor-induced anorexia and weight loss are mediated by the TGF-beta superfamily cytokine MIC-1. Nat. Med., 13, 1333–1340 (2007).

Tsai VW, Husainy I, Manandhar R, Lee-Ng KKM, Zhang HP, Harriott K, Jiang L, Lin S, Sainsbury A, Brown DA, Breit SN. Anorexia/cachexia of chronic diseases: A role for the TGF-beta superfamily cytokine MIC-1/GDF15. Journal of Cachexia, Sarcopenia and Muscle, 3, 239–248 (2012).

Welsh JB, Sapinoso LM, Kern SG, Brown DA, Liu T, Bauskin AR, Ward RE, Quinn MJ, Russell PJ, Sutherland RL, Breit SN, Mokaluk CA, Frierson HP Jr, Hampton GM. Large-scale delineation of secreted protein biomarkers overexpressed in cancer tissue and serum. Proc. Natl. Acad. Sci. U.S.A., 100, 3410–3415 (2003).

Tsai VW, Manandhar R, Jørgensen SB, Lee-Ng KKM, Zhang HP, Marquis CP, Jiang L, Husainy I, Lin S, Sainsbury A, Sawchenko PE, Brown DA, Breit SN. The anorectic actions of the TGF-beta superfamily cytokine MIC-1/GDF15 require an intact brainstem area postrema and nucleus of the solitary tract. PLOS ONE, 9, e100370 (2014).

Emmerson PJ, Wang F, Du Y, Liu Q, Pickard RT, Gonciarz MD, Cockshott P, Hamang MJ, Siddartha K, Ballman KK, Foltz LA, Flatt PM, Siegel R, Sloan JH, Mitchell PJ, Zhang BB, Donnellan M, Mahler S, Pryor K, Walsh BJ, Chen M, Ayupova DA, Lindhout DA, Higbee J, Solloway M, Haldankar R, Parsons T, Tang J, Shen WD, Alice A, Wang M, Laird T, Horner G, Chan J, McEntee M, Lopez M, Kutach A, Joo W, Gao Z, Fu D, To C, Mondal K, Li B, Kekatpure V, Vennema H, Car dashboardberno study. Circulation, 123, 2101–2110 (2011).

Breit SN, Carrero JJ, Tsai V, Yaghoutyfam N, Luo W, Kuffner T, Bauskin AR, Wu L, Jiang L, Barany P, Heimburger O, Marquis CP, Stattin P, Macia L, Lin S, Sainsbury A, Herzog H, Law M, Solloway M, Brown DA. Macrophage inhibitory cytokine 1 (MIC-1/GDF15) decreases food intake, body weight and improves glucose tolerance in mice on normal and obesi genic diets. PLOS ONE, 7, e43468 (2012).

Taylor RC, Dillin A. Aging as an event of protein stability collapse. Cold Spring Harb. Perspect. Biol., 3, a010440 (2011).

Ben-Zvi A, Miller EA, Morimoto RI. Collapse of proteostasis represents an early molecular event in Caenorhabditis elegans aging. Proc. Natl. Acad. Sci. U.S.A., 106, 14914–14919 (2009).

Hwee DT, Baehr LM, Philp AP, Baar K, Bodine SC. Maintenance of muscle mass and load-induced growth in Muscle RING Finger 1 null mice with age. Aging Cell, 13, 92–101 (2014).

Fujita Y, Taniguchi Y, Shinkai S, Tanaka M, Ito M. Secreted growth differentiation factor 15 as a potential biomarker for mitochondrial dysfunctions in aging and age-related disorders. Geriatr. Gerontol. Int., 16 (Suppl. 1), 17–29 (2016).

Wallent L, Zethelius B, Berglund L, Eggers KM, Lind L, Lindahl W, Wollert KC, Siegmann S, ADF-15 for prognostication of cardiovascular and cancer morbidity and mortality in men. PLOS ONE, 8, e76977 (2013).

Daniels LB, Clifton P, Laughlin GA, Maisel AS, Barrett-Connor E. Growth-differentiation factor 15 is a robust, independent predictor of 11-year mortality risk in community-dwelling older adults: The rancho bernardo study. Circulation, 123, 2101–2110 (2011).

López-Gallardo E, Ruiz-Pesini E, Artuch R, Montero R, Torner F, Nascimento A, Ortega C, Colomer J, Jiménez-Mallebrera C. Transcriptomic profiling of TK2 deficient human skeletal muscle suggests a role for the p53 signalling pathway and identifies growth and differentiation factor 15 as a potential novel biomarker for mitochondrial myopathies. BMC Genomics, 15, 91 (2014).

Chung HK, Ryu D, Kim KS, Chang JY, Kim YK, Yi HS, Kang SG, Choi MJ, Lee SE, Jung SB, Ryu MJ, Kim SJ, Kweon GR, Kim H, Hwang JH, Lee CH, Lee SJ, Wall CE, Downes M, Evans RM, Auwerx J, Shong M. Growth differentiation factor 15 is a myoamitochondria governing systemic energy homeostasis. J. Cell Biol., 216, 149–165 (2017).

Ito T, Yoshikawa N, Inui T, Miazakii N, Schaffer SW, Azuma J. Tissue delivery of tauire accelerates skeletal muscle senescence and leads to early death in mice. PLOS ONE, 9, e107409 (2014).

Ito T, Kimura Y, Uozumi Y, Takai M, Murakami S, Matsuoka T, Ueki K, Yoshiyama M, Ikawa M, Okabe M, Schaffer SW, Fujiyama Y, Azuma J. Taurine delivery caused by knocking out the taurine transporter gene leads to cardiomyopathy with cardiac arrhythmia. J. Mol. Cell. Cardiol., 44, 92–935 (2008).

Martinez-G, Duran-Aniotz C, Calbral-Miranda F, Vivar JP, Hetz C. Endoplasmic reticulum stress is a key mediator of aging in aging. Aging Cell, 16, 515–523 (2017).

Macia L, Tsai VW, Nguyen AD, Johnen H, Kuffner T, Shi YC, Lin S, Herzog H, Brown DA, Breit SN, Sainsbury A. Macrophage inhibitory cytokine 1 (MIC-1/GDF15) decreases food intake, body weight and improves glucose tolerance in mice on normal and obese diets. PLOS ONE, 7, e43468 (2012).

Taylor RC, Dillin A. Aging as an event of protein stability collapse. Cold Spring Harb. Perspect. Biol., 3, a010440 (2011).

Ben-Zvi A, Miller EA, Morimoto RI. Collapse of proteostasis represents an early molecular event in Caenorhabditis elegans aging. Proc. Natl. Acad. Sci. U.S.A., 106, 14914–14919 (2009).

Hwee DT, Baehr LM, Philp AP, Baar K, Bodine SC. Maintenance of muscle mass and load-induced growth in Muscle RING Finger 1 null mice with age. Aging Cell, 13, 92–101 (2014).

Fujita Y, Taniguchi Y, Shinkai S, Tanaka M, Ito M. Secreted growth differentiation factor 15 as a potential biomarker for mitochondrial dysfunctions in aging and age-related disorders. Geriatr. Gerontol. Int., 16 (Suppl. 1), 17–29 (2016).

Wallent L, Zethelius B, Berglund L, Eggers KM, Lind L, Lindahl W, Wollert KC, Siegmann ADF-15 for prognostication of cardiovascular and cancer morbidity and mortality in men. PLOS ONE, 8, e76977 (2013).

Daniels LB, Clifton P, Laughlin GA, Maisel AS, Barrett-Connor E. Growth-differentiation factor 15 is a robust, independent predictor of 11-year mortality risk in community-dwelling older adults: The rancho bernardo study. Circulation, 123, 2101–2110 (2011).

Breit SN, Carrero JJ, Tsai VW, Yagutsiam N, Luo W, Kuffner T, Bauskin AR, Wu L, Jiang L, Barany P, Heimburger O, Marquis CP, Macia L, Lin S, Sainsbury A, Herzog H, Law M, Stattin P, Brown DA. Macrophage inhibitory cytokine 1 (MIC-1/GDF15) decreases food intake, body weight and improves glucose tolerance in mice on normal and obese diets. PLOS ONE, 7, e43468 (2012).

Taylor RC, Dillin A. Aging as an event of protein stability collapse. Cold Spring Harb. Perspect. Biol., 3, a010440 (2011).