Review

Sarcopenia in Chronic Kidney Disease: Factors, Mechanisms, and Therapeutic Interventions

Hiroshi Watanabe,* a Yuki Enoki, b and Toru Maruyama a

a Department of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Kumamoto University; 5–1 Oe-honmachi, Chuo-ku, Kumamoto 862–0973, Japan; and b Division of Pharmacodynamics, Keio University Faculty of Pharmacy; Tokyo 105–8512, Japan.

Received June 25, 2019

Chronic kidney disease (CKD), a chronic catabolic condition, is characterized by muscle wasting and decreased muscle endurance. Many insights into the molecular mechanisms of muscle wasting in CKD have been obtained. A persistent imbalance between protein degradation and synthesis in muscle causes muscle wasting. During muscle wasting, high levels of reactive oxygen species (ROS) and inflammatory cytokines are detected in muscle. These increased ROS and inflammatory cytokine levels induce the expression of atrogenes such as atrogin-1 and muscle ring factor 1, members of the muscle-specific ubiquitin ligase family. Impaired mitochondrial function also contributes to reducing muscle endurance. The increased protein-bound uremic toxin, parathyroid hormone, glucocorticoid, and angiotensin II levels that are observed in CKD all have a negative effect on muscle mass and endurance. Among the protein-bound uremic toxins, indoxyl sulfate, an indole-containing compound has the potential to increase muscle atrophy through stimulating ROS-mediated myostatin and atrogenes expression. Indoxyl sulfate also impairs mitochondrial function. Some potential therapeutic approaches based on the muscle wasting mechanisms in CKD are currently in the testing stages.

Key words muscle wasting; myostatin; mitochondria; oxidative stress; uremic toxin; indoxyl sulfate

1. INTRODUCTION

Skeletal muscle atrophy, referred to as sarcopenia, and decreased exercise endurance are frequently observed in chronic kidney disease (CKD) patients and are correlated with the risk of morbidity and mortality in such patients. 1–5 Therefore, improving physical performance is an important factor for improving the prognosis of CKD patients. In chronic catabolic conditions such as CKD, persistent imbalances between protein degradation and synthesis result in a loss of muscular protein mass (Fig. 1). Impaired mitochondrial function also contributes to reducing muscle endurance. This review explores the recent available evidence for the molecular mechanism of muscle wasting, especially uremic toxin-induced muscle wasting, and potential therapeutic agents that might be used to counteract muscle atrophy in CKD.

2. MOLECULAR MECHANISM OF MUSCLE ATROPHY IN CKD

2.1. Protein Degradation in Muscle

2.1.1. Atrogenes: Atrogin-1, Muscle Ring Factor 1, and Autophagy-Related Genes

A balance between protein degradation and synthesis is important for the maintenance of muscle mass. Therefore, a decrease in muscle mass is attributed to either an increase in protein degradation or a decrease in protein synthesis. Several molecular mechanisms on CKD-induced skeletal muscle atrophy have been proposed so far. In fact, multiple intracellular signaling pathways increase the expression of atrogenes such as atrogin-1 and muscle ring factor 1 (MuRF-1), the member of the muscle-specific ubiquitin ligase family, in addition to autophagy-related genes. 6–8 (Fig. 1). The increased expression of these atrogenes induces protein degradation via the ubiquitin–proteasome system (UPS) and autophagy. In CKD condition, increased oxidative stress, inflammation, protein-bound uremic toxins, parathyroid hormone, glucocorticoid, and angiotensin II, and defective insulin signaling can initiate the above pathways. 6–10 Hemodialysis procedures can also reduce protein synthesis and stimulate protein degradation. 11

2.1.2. Myostatin and Transforming Growth Factor-β (TGF-β)

Myostatin, an autocrine inhibitor of muscle growth, is produced predominantly in skeletal muscle. 12,13 Myostatin binds to the activin A receptor type IIB (ActRIIB) on muscle cell, followed by activation of the downstream signaling Smad2/3 14,15 (Fig. 1). In the skeletal muscle of CKD patients, myostatin expression was upregulated. 16 The muscular myostatin was also increased in five-sixths nephrectomized mice (CKD mice) as well as CKD patients 17 and that the administration of an anti-peptide against myostatin to these mice suppressed the muscle loss. 18 Myostatin expression is enhanced by oxidative stress and inflammation 10,19–21 through the forkhead box protein O (Foxo), nuclear factor-kappaB (NF-κB), 22 and Smad2/3.

TGF-β also functions as a potent inducer of muscle loss. The administration of TGF-β induced muscle atrophy through atrogin-1 induction. 23 TGF-β binds to TGF-β type I/II receptors, that activate the Smad2/3 signaling pathways to induce atrogenes (Fig. 1).

2.2. Protein Synthesis in Muscle: Insulin/Insulin-Like Growth Factor-I–Akt–Mammalian Target of Rapamycin Signaling and Foxo Activation

The insulin or insulin-like growth factor (IGF)-1–phosphatidylinositol 3-kinase (PI3K)–
Akt pathway plays important roles in the increase of skeletal muscle mass by increasing muscle protein synthesis via the mammalian target of rapamycin (mTOR) and by decreasing protein degradation via the inactivation of the Foxo.24–27) Muscle atrophy was increased under conditions where insulin responsiveness was impaired and atrogin-1 activity was increased.28) Sandri et al. found that a decrease in Akt activity led to the induction of Foxo transcription factors and atrogin-1. In addition, IGF-1 treatment or the Akt overexpression suppressed the expression of Foxo and atrogin-1.29) In this scenario, the expression of atrogin-1 and MuRF-1 are suppressed by Akt via the inactivation of Foxo, a negative regulator of transcriptional factors for atrogenes.25,28,29)

2.3. Mitochondria In skeletal muscle, exercise capacity and mitochondrial function are mutually connected.30) The amount of mitochondria is controlled by mitochondrial biosynthesis and degradation.31,32) In the mice with early-stage CKD model, intramuscular mitochondria and running distance were decreased and those were correlated with oxidative stress and inflammatory responses.33) These oxidative stress and inflammatory responses decreased the expression of the peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α), a master regulator of mitochondrial biosynthesis, and increased autophagy, a mitochondria degradation system. The PGC-1α overexpression showed resistance to muscle atrophy in mice with denervation or fasting.34) Similarly, the PGC-1α overexpression in mice ameliorated muscle atrophy, leading to an extended life span.35) In skeletal muscle of CKD patients, the numbers of mitochondria were decreased.36) In addition, exercise increased the mitochondria content in the muscle of CKD patients. Therefore, a decrease in the number of mitochondria in muscle appears to play a critical role in muscle endurance in CKD patients.

3. MOLECULAR MECHANISM OF UREMIC TOXIN-INDUCED MUSCLE WASTING

3.1. Oxidative Stress and Inflammation During muscle wasting, abnormally high levels of reactive oxygen species (ROS) and inflammatory cytokines are produced in skeletal
An increase in ROS-induced tumor necrosis factor (TNF)-α stimulates myostatin expression through NF-κB pathway, which further stimulates myostatin expression accompanied by interleukin (IL)-6 release in muscle. These signaling further increases ROS levels through reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Therefore, increased ROS production induces a negative feed-forward loop which further increases myostatin (Fig. 2).

Inflammatory cytokines such as TNF-α and IL-6, which were increased in the muscle tissue of CKD mice, cause muscle atrophy. The myostatin inhibition decreased the plasma levels of these cytokines. In addition, the administration of TNF-α and IL-6 to mice induced muscle atrophy, while this muscle atrophy was suppressed by the neutralization of these cytokines. TNF-α also increased myostatin expression, which further accelerates muscle catabolism. Similar to myostatin, oxidative stress and inflammatory cytokines also stimulates atrogin-1 expression. These findings concluded that the muscle atrophy is mutually linked with myostatin, atrogenes, oxidative stress, and inflammation.

3.2. Uremic Toxins
Accumulated uremic toxins in the body under CKD conditions exert biological activities. Among the uremic toxins, the protein-bound uremic toxins, such as indoxyl sulfate, p-cresyl sulfate, indole acetic acid, hippuric acid, kynurenic acid, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid, etc. has been focused of the difficulty associated with their removal by hemodialysis due to their strong binding to albumin. These protein-bound uremic toxins contribute to the progression of CKD and CKD complications such as cardiovascular damage. These renal toxicity and cardiovascular damage were stimulated by enhancing oxidative stress and inflammation. In addition, a high-protein diet not only exacerbates impaired renal function but also reduces exercise endurance in CKD mice, which is accompanied by the increased uremic toxins production. These evidences hypothesize that protein-bound uremic toxins play an essential role in muscle atrophy and reduced endurance.

Molecular mechanisms of the harmful actions of protein-bound uremic toxins have been proposed so far. For example, these toxins are taken up by the cells through an organic anion transporter (OAT). Then, these toxins show their toxicity via NADPH oxidase, which leads to the increase of ROS and inflammatory cytokines. In addition, indole-containing toxins, such as indoxyl sulfate, act as aryl hydrocarbon receptor (AHR) ligand and exert their toxicity via AHR. Interestingly, AHR also works as one of the components of the ubiquitin ligase complex. We have recently demonstrated that, among the protein-bound uremic toxin, indoxyl sulfate contributes to skeletal muscle loss.

3.3. Distribution of Indoxyl Sulfate in Muscle Tissue
OATs such as OAT1 and OAT3 take up indoxyl sulfate into cells. Western blotting analyses showed that the mouse Oat1 and Oat3 are expressed in C2C12 mouse myoblast cells. In addition, we found that exogenously administered indoxyl sulfate distributed to skeletal muscle. At the same time, the pattern of the immunostaining image of indoxyl sulfate in skeletal muscle was similar to that of ROS production. Therefore, indoxyl sulfate induces ROS production in skeletal muscle.
muscle in vivo (Fig. 4).

3.4. Redox Properties of Indoxyl Sulfate in Skeletal Muscle Indoxyl sulfate inhibits the proliferation of C2C12 myoblast cells. In addition, indoxyl sulfate causes increased ROS production and inflammatory cytokine expression (TNF-α, IL-6, and TGF-β1) in C2C12 cells. These also enhance the expression of myostatin and atrogin-1. But these effects induced by indoxyl sulfate were prevented by the presence of an antioxidant, OAT, inhibitor, AHR inhibitor, and small interfering RNA (siRNA) for AHR. The chronic administration of indoxyl sulfate to half-nephrectomized mice reduced their body weights and muscle weight. In skeletal muscle of these mice, indoxyl sulfate stimulates myostatin and atrogin-1 expression after increasing ROS and inflammatory cytokines production.10) These data were supported by Changchien et al.,68) who demonstrated the importance of the ROS-extracellular signal-regulated kinase (ERK) axis and c-Jun N terminal kinase (JNK)-atrogin-1 regulation by indoxyl sulfate in indoxyl sulfate-induced myotube atrophy. Indoxyl sulfate impaired mitochondrial function by decreasing the PGC-1α and autophagy induction67) (Fig. 4).

Sato et al. reported that, in their clinical research, an association between indoxyl sulfate level in plasma and muscle mass was seen in patients with CKD.69) Those data also suggested that indoxyl sulfate could be a pathogenic factor for muscle atrophy in CKD.

3.5. Effect of p-Cresyl Sulfate on Insulin Signaling in Skeletal Muscle Koppe et al. demonstrated that when mice are treated with p-cresyl sulfate, insulin signaling is altered in skeletal muscle, where p-cresyl sulfate inhibited insulin-stimulated glucose uptake and decreased insulin signaling pathways through the activation of the ERK kinase50) (Fig. 5). Regarding the downstream pathway of insulin signaling, p-cresyl sulfate suppressed the insulin-induced phosphorylation of Akt. Since indoxyl sulfate had no effect on Akt phosphorylation,10) the effects of indoxyl sulfate or p-cresyl sulfate on muscle atrophy appear to be mutually independent (Figs. 4, 5).

3.6. Parathyroid Hormone Contributes to Muscle Atrophy via Parathyroid Hormone Receptor Expressed in Fat Tissue: Kidney-Fat-Muscle Crosstalk Kir et al. demonstrated that the parathyroid hormone (PTH), a middle-molecule uremic toxin, is involved in stimulating the expression of thermogenic genes, such as UCP1, in five-sixths nephrectomized CKD mice.9) In this mouse model, the expression of the atrogin-1, MuRF1, and myostatin genes was increased in gastrocnemius muscle tissue, whereas IGF-1 expression was decreased. Interestingly, they also showed that the loss of PTH receptors in fat tissue blocked the upregulation of thermogenic genes and prevented muscle atrophy. These data indicate that PTH/PTH receptor signaling in fat tissue is an important player in muscle atrophy in CKD (Fig. 6).

4. OTHER INITIATING FACTORS CONTRIBUTE TO THE MUSCLE ATROPHY IN CKD

4.1. Glucocorticoids Increased circulating glucocorticoids are associated with muscle atrophy. Muscle-specific glucocorticoid receptor knockout mice were resistant to glucocorticoid-induced muscle atrophy,71) suggesting that the glucocorticoid receptor is essential for muscle atrophy induced by glucocorticoids. Several reports showed that glucocorticoids increase myostatin expression,72–75) thereby inducing protein breakdown by enhancing atrogin-1 and MuRF1 expression and decreasing protein synthesis by inhibiting the mTOR pathway. In particular, in the case of the IGF-1–PI3K–Akt–mTOR pathway, glucocorticoids were found to inhibit IGF-1 production76,77) and accelerate the degradation of insulin receptor growth factor (IRS-1), followed by reduced PI3K activity.78,81) Frost and Lang showed that the constitutively activated form of Akt suppressed the negative effects of glucocorticoids on protein synthesis82) and this was also demonstrated by other group to suppress glucocorticoids-induced muscle loss.29) Glucocorticoids also cause an increase in Foxo gene expression,73,88) It therefore appears that glucocorticoid receptors and Foxo synergistically contribute to the upregulation of atrogin expression.84) Muscle atrophy induced by glucocorticoid is characterized by fast-twitch (type II muscle fiber) atrophy.85) On the other hand, the administration of glucocorticoid paradoxically exerted a positive effect on muscle function, probably due to suppressing inflammatory cytokine expression.86)
tions, circulating IGF-1 levels were reduced by about 30% in angiotensin II-treated rats. Zhang et al. also demonstrated that the infusion of angiotensin II increased the levels of plasma IL-6 and its hepatic production. In addition, the infusion of angiotensin II stimulates the suppressor of cytokine signaling (SOCS3) in muscle, which leads to a loss of the insulin receptor substrate 1 (IRS-1), thus impairing insulin/IGF-1 signaling. Benigni et al. reported that the mouse homologue of angiotensin II type 1 (AT1) knockout mice (agtr1a−/−) showed a decrease in ROS production and an increase in the number of mitochondria. In addition, the mice had a prolonged life span. The administration of irbesartan, an AT1 receptor blocker, improved muscle repair through the downregulation of the aging associated Cis-Wnt/β-catenin signaling pathway. These data indicate that angiotensin II can stimulate muscle atrophy through a defect in insulin/IGF-1 signaling and an inflammatory mechanism via an AT1 receptor.

5. POTENTIAL THERAPEUTIC INTERVENTIONS FOR CKD-ASSOCIATED MUSCLE WASTING

5.1. Blocking Myostatin-ActRIIB Signaling Myostatin is a negative regulator of skeletal muscle mass, which is known to signal via the ActRIIB receptor on skeletal muscle, thereby inducing muscle wasting. Morvan et al. have recently shown that bimagrumab, acting as a human dual-specific anti-ActRIIA/ActRIIB antibody, neutralizes muscle atrophy. The activin decoy receptor ActRIIB also prevents skeletal muscle pathophysiology. Endogenous circulating proteins such as follistatin and follistatin-like proteins are known to inhibit the binding of myostatin to ActRIIB. Lee and McPherron reported that transgenic mice expressing high levels of follistatin showed an increased muscle mass. Chang et al. also demonstrated that the overexpression of muscle-specific follistatin enhanced skeletal muscle growth. Follistatin gene therapy against sporadic inclusion body myositis or facioscapulohumeral muscular dystrophy improved functional outcomes such as the distance traveled in a 6-min walk test. Follistatin delivery systems such as nanoparticles, Fc fusion systems, etc. are under development in clinical settings. In addition, an anti-myostatin peptibody that binds myostatin or blocking its receptor is also under development. These data suggest that molecules that block myostatin-ActRIIB signaling would be potentially useful for enhancing muscle growth.

5.2. AST-120 AST-120 is prescribed for CKD patients to inhibit the accumulation of indoxyl sulfate. The administration of AST-120 to CKD mice recovered the exercise capacity, muscle weight and mitochondrial function via decreasing oxidative stress.

5.3. L-Carnitine In CKD patients, restricted protein intake, decreased biosynthesis of L-carnitine, and the removal of L-carnitine by dialysis lead to L-carnitine deficiency. L-Carnitine deficiency decreases muscle power, while supplementation of L-carnitine is effective in treating myopathy and muscle loss. In CKD mice, L-carnitine treatment ameliorates muscle atrophy and exercise capacity. This can be attributed to the prevention of impaired mitochondrial function and decreased muscle fibers.

5.4. Dipeptidyl Peptidase-4 Inhibitor Teneligliptin, an inhibitor of dipeptidyl peptidase-4 (DPP-4), has potential for the treatment of CKD-induced muscle wasting. The DPP-4 enzyme degrades the incretin hormones such as GLP-1. GLP-1 increased PGC-1α expression and mitochondrial membrane potential. Administration of teneligliptin ameliorated impaired mitochondrial function in mice fed a high-fat diet. GLP-1 also ameliorated insulin resistance through the PI3K-Akt signaling pathway in skeletal muscle. Teneligliptin also acts as a direct radical scavenger. Using human proximal tubular cells, a DPP-4 inhibitor showed the cell protective activity without the action of GLP-1. Taking these findings into consideration, a DPP-4 inhibitor may exert cytoprotective activities not only inhibiting the degradation of GLP-1 but also via its direct action.

5.5. Ghrelin Tamaki et al. reported that the administration of acylated ghrelin to five-sixths nephrectomized CKD mice increased muscle mass and muscular mitochondrial content through increasing PGC-1α expression. It was also reported that the nonpeptidergic ghrelin receptor agonist counteracts cachectic body weight loss under inflammatory conditions.

5.6. MicroRNA (miRNA) Increased miR27a/b was reported to negatively regulate the expression of myostatin. Wang et al. investigated the role of miR-23a and miR-27a in the regulation of muscle mass. The injection of an adenovirus encoding miR-23a and miR-27a or the overexpression of miR-23a and miR-27a in CKD mice suppressed muscle loss by increasing Akt phosphorylation. miR1 is a muscle-specific microRNA that induces muscle atrophy by regulating heat-shock protein 70. The antagonism of miR1 may be beneficial during muscle atrophy. Hu et al. reported that low-frequency electrical stimulation ameliorates CKD-induced muscle atrophy by upregulating the IGF-1 signaling pathway through decreasing the expression of miR1 and miR206. Interestingly, low-frequency electrical stimulation induces the activation of M2 macrophages.

6. CONCLUSION

This review summarizes the available evidence for the molecular mechanism of muscle wasting, especially uremic toxin-induced muscle wasting in CKD. It is noteworthy that oxidative stress and inflammation appear to be contributors to the muscle atrophy caused by a decrease in muscle mass and mitochondrial dysfunction. Increased levels of protein-bound uremic toxins such as indoxyl sulfate, a middle-molecule uremic toxin PTH, glucocorticoids, and angiotensin II also contribute to this type of muscle atrophy and reduced muscle endurance in CKD. These data point to the importance of developing potential therapeutic agents for counteracting the muscle atrophy associated with CKD.

Acknowledgments We are grateful to Professor Masafumi Fukagawa, Division of Nephrology, Endocrinology and Metabolism, Tokai University School of Medicine, Kanagawa, Japan; We also grateful to Dr. Motoko Tanaka and Dr. Kazutaka Matsushita, Department of Nephrology, Akebono Clinic, Kumamoto, Japan for their valuable advices. Our work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (KAKENHI 25460190; 16H05114), Research Foundation for Pharmaceutical Sciences, Japan, and Nakatomi Foundation.
Conflict of Interest  The authors declare no conflict of interest.

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