EDITORIAL

Going micro in CKD-related cachexia

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Muscle wasting in chronic kidney diseases (CKDs), sometimes referred to as protein energy wasting or cachexia, has been widely studied, yet much remains to be understood about the underlying pathophysiology. CKD-related changes in cell signaling disrupt proteostasis by activating muscle proteolysis [e.g. ubiquitin–proteasome system (UPS), autophagy and caspase-3] and negatively impacting protein synthesis and muscle regeneration. Muscle mass is controlled by a variety of signaling pathways, the most prominent being the insulin-GF-1)/Phosphatidylinositide 3-kinase (PI3K)/Protein Kinase B (Akt) pathway, which provides a point of intersection for protein synthesis, protein degradation and myogenesis. Akt phosphorylates and activates mTOR, which in turn stimulates key components of the protein synthesis and myoblast differentiation machineries. In contrast, Akt phosphorylates and inhibits the Forkhead box class O (FOXO) transcription factors, which increase the expression of several atrophy-inducing proteins including atrogin-1/MAFbx and Muscle RING finger 1 (MURF1) [1]. Other important signaling pathways include the Janus kinase/signal transducers and activators of transcription (JAK/STAT) and Activin receptor-IIB (ActRIIB)/SMAD pathways, which mediate the effects of cytokines [e.g. interleukin-6 (IL-6)] and the muscle-specific autocrine factor, myostatin (also called GDF-8), respectively [2].

In the past decade, we have begun to understand how changes in cell signaling and metabolic pathways are coordinately regulated by microRNAs (miRNAs), small RNAs that bind to complimentary sequences in messenger RNA (mRNA) 3'-untranslated regions. This interaction can either facilitate mRNA degradation or silence translation of the mRNA into protein [3, 4]. Emerging evidence indicates that miRNAs play important roles in the development and repair of muscle as well as the normal maintenance of mature muscle fibers. Because of their small 'functional' or seed unit of six to eight nucleotides, miRNAs typically target multiple proteins, and frequently, the targets are in the same signaling pathway. This is particularly true for the IGF-1/PI3K/Akt and myostatin/SMAD pathways, both of which modulate key steps in protein synthesis and degradation. Expression of YY1 and other regulators of muscle

satellite cell function and differentiation are also modulated by miRNAs [5]. In this issue of *Nephrology Dialysis Transplantation*, Robinson *et al.* [6] review how miRNAs contribute to normal muscle development, the control of muscle protein turnover in CKD and CKD-associated interorgan cross talk. They also discuss how miRNAs represent future potential therapeutic agents for treating muscle pathologies [6].

Alterations in miRNAs have been linked to dysfunctional proteostasis in skeletal muscle during CKD [7–10]. MiR1, miR-133 and miR-206 are increased in CKD and negatively regulate the early steps of the IGF-1/PI3K/Akt pathway. In contrast, miR-23a, miR-27a and miR-486 are decreased and modulate cell signaling mediators and atrogenes (i.e. atrogin-1/MAFbx). Interestingly, miR-23a and miR-27a, along with miR-24a, are located in a single cluster on the chromosome (chromosome 19 in humans and chromosome 8 in mice) [9, 11]. They are transcriptionally controlled by a single promoter and transcribed as a single RNA that is processed into individual miRNAs. miR-23a and miR-27a target muscle-specific atrophy-inducing enzymes associated with the UPS. They also target suppressors of the IGF-1/PI3K/Akt pathway including myostatin, a proteostasis-disrupting myokine member of the Transforming Growth Factor-β (TGF-β) superfamily that activates the SMAD pathway. In addition to direct actions on muscle, myostatin impairs muscle precursor cell proliferation and the regenerative capacity of muscle, which is decreased in CKD. miR-133 similarly impacts myoblast proliferation differentiation through actions on IGF-1 and IGF-1R [3].

Although the focus of the article by Robinson *et al.* [6] is largely on the miRNAs that disrupt proteostasis, it is important to be reminded how other signals such as mitochondrial dysfunction and inflammation play prominent roles in CKD-related cachexia. Inefficiencies in mitochondrial function lead to skeletal muscle weakness and decreased exercise capacity in CKD patients [12] and animals [13]. In CKD, reduced mitochondrial number and mass, as well as mitochondrial damage and dysfunction, are linked to atrophy-inducing responses, including increased oxidative stress and autophagy/mitophagy. Reduced Peroxisome proliferator-activated receptor gamma

coactivator 1-α (PGC-1α) and an increase in BNIP3 lead to reduced mitochondrial biogenesis and ATP production [13]. PGC-1α is a key transcriptional coactivator that is important for mitochondrial biogenesis [14] and BNIP3 is a pro-apoptotic protein localized in the outer mitochondrial membrane. It regulates the opening of the mitochondrial permeability pore and is linked to intrinsic apoptosis [15]. Consistent with an increase in BNIP3, Du et al. [16] found that elevated caspase-3 activity in muscle of CKD rats contributed to their excessive proteolysis. In other studies, Su et al. [13] reported that the level of BNIP3 was linked to the deterioration of mitochondrial function and decreased ATP production. Using in vivo magnetic resonance and optical spectroscopy, Roshanravan et al. [17] found that resting oxygen consumption was elevated and mitochondrial coupling was lower in early CKD patients. Tamaki et al. [18, 19] also reported a decline in muscle mitochondria and exercise endurance occurs early in CKD mice. These findings, when coupled with the CKD-induced decrease in PGC-1α, are compelling evidence that mitochondrial dysfunction contributes to CKD cachexia.

Inflammation is another prominent mediator for the wasting process in CKD. Production of inflammatory cytokines [i.e. tumor necrosis factor (TNF)-α and IL-6] by myofibers and other cell types (e.g. macrophages) is increased in CKD and they act in a paracrine/endocrine fashion on both myofibers and satellite cells [20]. Inflammatory cytokines (e.g. TNF-α) activate nuclear factor-κB, which in turn induces transcription of key atrogenes in myofibers and inhibits the MyoD differentiation factor in myoblasts [20, 21]. TNF-α also increases myostatin expression and induces apoptotic signaling, both of which exacerbate the muscle catabolism processes already described. IL-6 also is a causative agent of CKD cachexia, although its role in the muscle may be somewhat variable and situational. There are substantial data indicating that IL-6 induces muscle wasting [22]. Others have reported that increased IL-6 facilitates local infiltration of macrophages, which upregulate locally produced IGF-1 and limited muscle loss in CKD mice [23]. In humans, an increase in IL-6 was associated with increased muscle protein synthesis during hemodialysis [24].

Exercise is one of a few interventions shown to help maintain muscle mass while also slowing or improving kidney function in CKD [25, 26]. Recent studies of the mechanisms underlying the effects of exercise in CKD have revealed an interesting and powerful communications network between muscle and other organs, including kidney (i.e. cross talk). Cross talk between organs provides advantages or disadvantages, depending on the mediators involved. This interorgan communication typically occurs via production and release of proteins, lipids, metabolites and/or nucleic acids. In one example of protein exchange, Peng et al. [27] uncovered a link between the CKD-related reduction of the exercise-responsive PGC-1α transcription coactivator in muscle and irisin, a myokine that impacts oxidative metabolism in muscle and other organs [27]. Using a folate model of acute kidney injury, they found less kidney damage in mice that overexpress PGC-1α in muscle only versus normal controls. The group then used recombinant irisin to confirm that the myokine was responsible

for the improvement in mitochondrial respiration, energy metabolism and reduced fibrosis in damaged tubule cells.

As discussed by Robinson et al. [6], there is compelling evidence for a role of miRNAs in the beneficial effects of exercise. In a recent study, acupuncture with low-frequency electrical stimulation (Acu/LFES) ameliorated hind limb muscle loss due to CKD in mice [23]. The mechanism involved the upregulation of the IGF-1/PI3K/Akt pathway in both myofibers and muscle satellite cells [23]. In a follow-up study, miR-181 was increased in hind limb muscles and serum exosomes following Acu/LFES [28]. Surprisingly, the Acu/LFES procedure also increased renal blood flow in CKD mice and the response was limited by blocking exosome secretion. The group then demonstrated that miR-181 targets angiotensinogen and that renal angiotensinogen protein was lower in cachexic mice following Acu/LFES. In an unrelated study, resistance exercise in the form of synergistic ablation attenuated CKD-induced muscle loss and increased the levels of miR-23a and -27a in muscles. Using a streptozotocin model of diabetic muscle atrophy and reduced renal function, the investigators found that overexpression of miR-23 and miR-27 in muscle improved renal function as evidenced by a reduction in blood urea nitrogen (BUN) and reduced levels of renal fibrosis [29]. As in the Acu/LFES studies, the mechanism for the improvement in renal function and fibrosis involved exosome-mediated delivery of miR-23a and miR-27a to the kidney. Together with the irisin study by Peng et al. [27], these findings provide some of the strongest evidence to date for muscle–kidney cross talk.

There are challenges with using miRNAs as therapeutic agents. Free RNAs are rapidly degraded and miRNAs need to be targetable to minimize undesirable side effects. Encapsulation of miRNAs into extracellular vesicles makes them more stable and provides a means to target them. Wang et al. developed a novel approach to this challenge [7, 10]. They maintained muscle satellite cells in culture and used molecular biological tools to provide the cells with a desired miRNA and to produce exosomes with cell-targeting peptides embedded in their membranes [7, 10]. These 'engineered' exosomes were collected from the media of the satellite cells and then injected into a single hind limb muscle of mice with unilateral ureteral obstruction-induced kidney injury and muscle atrophy. The therapy reduced both muscle loss and renal fibrosis. Fluorescent tags from the collected and labeled exosomes were found in both uninjected muscles and kidneys of treated mice. This approach has the potential to serve as a foundation for individualized treatments in patients using their own satellite cells.

In conclusion, Robinson et al. [6] provide a thoughtful overview of the emerging role of miRNAs in skeletal muscle wasting of CKD. In some cases, inconsistencies in study outcomes diminish our ability to draw strong conclusions. For example, levels of total and specific miRNAs in CKD patient serum have been reported to either increase or decrease [30, 31]. Outcome variability may result from the use of different analytic methodologies or different underlying etiologies. These discrepancies underscore the need for more comprehensive studies in cell models, animals and especially in patients to validate and

confirm how specific miRNAs change with CKD. Such studies will better enable us to fully evaluate the therapeutic potential of miRNAs as a modality to limit the progression of CKD and the associated cachexia.

CONFLICT OF INTEREST STATEMENT

None declared. The authors have nothing to disclose. The text of this editorial has not been published previously.

(See related article by Robinson *et al.* Skeletal muscle wasting in chronic kidney disease: the emerging role of microRNAs. *Nephrol Dial Transplant* 2020; 35: 1469–1478)

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