Current Perspective

Skeletal Muscle Is an Endocrine Organ

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Abstract. Skeletal muscle plays a key role in postural retention as well as locomotion for maintaining the physical activities of human life. Skeletal muscle has a second role as an elaborate energy production and consumption system that influences the whole body’s energy metabolism. Skeletal muscle is a specific organ that engenders a physical force, and exercise training has been known to bring about multiple benefits for human health maintenance and/or improvement. The mechanisms underlying the improvement of the human physical condition have been revealed: skeletal muscle synthesizes and secretes multiple factors, so-called as myokines, and these muscle-derived factors exert beneficial effects on peripheral and remote organs. In this short review, we focus on the third aspect of skeletal muscle function — namely, the release of multiple types of myokines, which constitute a broad network for regulating the function of remote organs as well as skeletal muscle itself. We conclusively show that skeletal muscle is one of the endocrine organs and that understanding the mechanisms of production and secretion of myokines may lead to a new pharmacological approach for treatment of clinical disorders.

Keywords: skeletal muscle cell, myokine, exercise

1. Introduction

Skeletal muscle plays a key role in postural retention as well as locomotion for maintaining the physical activities of human life. Skeletal muscle has a second role as an elaborate energy production and consumption system that influences the whole body’s energy metabolism. In this short review we will focus on the third aspect of skeletal muscle, whereby it constitutes a network for regulating the function of remote organs as well as skeletal muscle itself by the release of multiple types of myokines.

2. Skeletal muscle and adipokines

In recent years, significant advances have been made in understanding the pathophysiological functions of skeletal muscle and the involvement of functional abnormalities of skeletal muscle in various disorders (1). Impairment of insulin resistance, together with β-cell secretary function defects, is a key contributor to the occurrence of Type 2 diabetes mellitus (DM). Relevant metabolic syndrome (MetS) and its associated risk factors for cardiovascular disorders are currently matters of grave concern. Weight reduction has been proposed as an efficacious method to reduce the onset of these disorders by normalizing impaired fasting blood glucose and preventing the subsequent complications. Diet and exercise (i.e., lifestyle interventions) have been recognized as procedures for controlling the progression (2). Skeletal muscle is a key organ for oxidation of lipids and carbon hydration in the process of energy production and/or consumption (3). To establish strategies to prevent the progression of DM as well as MetS, we noticed that it is essential to understand the health status of skeletal muscle (4). For successful management of glucose tolerance and lipid metabolism, skeletal muscle must maintain its normal functional activity and ability to comply with demands from the whole body.

Recently, adipose tissue was recognized as an endocrine organ due to its secretion of diverse types of substances related to obesity-linked disorders (5). The principal function of these molecules, generally recognized as adipokines, was to regulate the lipid as well
as glucose metabolism, and thus skeletal muscle was a major target. Consequently, the provisional concept that skeletal muscle acts as a silent contributor to realize an order from the adipokines was introduced.

3. Exercise and the concept of myokine

Exercise training, or under various types of conditions (i.e., aerobic or anaerobic) has been known to bring about multiple benefits for human health maintenance and/or improvement. The mechanisms underlying the improvement of the human physical condition have recently been revealed: skeletal muscle synthesizes and secretes multiple factors, and these muscle derived-factors exert beneficial effects on peripheral and remote organs. At present, these skeletal muscle-derived bioactive materials are called myokines, and the presence of complicated and divergent crosstalk with other organs has been elucidated (6). Scientists now understand that skeletal muscle is not a simple passive effector but rather an endocrine organ that produces and releases various types of biological molecules (myokines) toward the targeted organs via the circulation as well as for itself in paracrine fashion.

Skeletal muscle is composed of multiple types of cells including fibroblasts, pericytes, adipocytes, motor neurons, and connective tissues. Among these skeletal muscle–constituting cells, not only myocytes (muscle fiber) but also pericytes including satellite cells have been reported to possess signal interaction with surrounding cells that relate to bioactive factor secretion (7). It still needs to be clarified whether myokines are derived from skeletal myocytes or other cells, and whether or how the cells utilized in such experiments are differentiated. Moreover, the observed myokine level change could be determined by a particular experimental condition or could vary according to the source of the sample used in the studies. The term “myokine” may refer to factors secreted by contracting skeletal myocytes, the increase of which in the circulating blood stream is detected in response to muscle contraction. To overcome the limitation of in vitro experimental conditions, usually performed under non-contracting cultured cells, we designed an original, pressure-loading apparatus and reported that this system enabled us to reproduce the intramuscular pressure increments that occur during human exercise in cultured skeletal muscle cells (8).

4. Myokines

To address skeletal muscle function as an endocrine organ, we performed in vitro experiments with differentiated human cultured skeletal muscle cells (Huskmc) under pressurized condition to reproduce the intra-muscular pressure elevation that occurs during exercise. PCR-array analyses profiled the expression of 84 genes related to growth factors, resulting in a significant mRNA expression change in multiple factors including the members of the transforming growth factor-β (TGF-β) superfamily and related factors as well as interleukins (Table 1) (unpublished data). The TGF-β superfamily and related factors are known as the first candidate molecules for functioning as a myokine in skeletal muscle.

4.1. TGF-β superfamily and related molecules

The TGF-β superfamily involves multiple structurally related proteins. The TGF-β family and associated factors are known to induce biological signals that regulate cell growth, regeneration, differentiation, transformation, and death in skeletal muscle. Previous reports have demonstrated the complicated interaction between each factor based on positive and negative ligands for signal transduction (9). Intereelationships among the TGF-β superfamily and related molecules mentioned in this review are illustrated in Fig. 1.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Fold change</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>INHA</td>
<td>Inhibin, alpha</td>
<td>6.6877</td>
<td>0.044</td>
</tr>
<tr>
<td>BMP4 (BMP-2b)</td>
<td>Bone morphogenetic protein 4</td>
<td>2.7844</td>
<td>0.008</td>
</tr>
<tr>
<td>IL11</td>
<td>Interleukin 11</td>
<td>0.2687</td>
<td>0.00001</td>
</tr>
<tr>
<td>IL1B</td>
<td>Interleukin 1, beta</td>
<td>0.052</td>
<td>0.00009</td>
</tr>
</tbody>
</table>

Huskmcs were further cultured for 5 days to promote differentiation in skeletal muscle cell differentiation medium. Differentiated Huskmcs were cultured on 60-mm dishes and were placed in a humidified pressure-loading apparatus. Then cells were exposed to an atmospheric pressure of 200 mmHg, at 37°C, in an atmosphere containing 5% CO₂ for 120 h to reproduce intramuscular pressure increments during human exercise. PCR-array analyses (RT² Profiler™ PCR Array Human Growth Factors) were performed on triplicate cDNA samples synthesized by total RNA. mRNA level changes on pressurized samples were expressed as a ratio (fold change) against non-pressurized samples. P-values indicate the statistical significance of differences between groups.
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4.1.1. Myostatin

Among over 50 members of the TGF-β structurally related superfamily, myostatin, also known as growth/differentiation factor-8, has been classified into the decapentaplegic-Vg-related (DVR) subfamily and was the first ligand identified as a myokine in rodents (10). Myostatin mRNA expression was observed in cultured mouse myogenic skeletal muscle cells, and the serum concentration was also quantified in both healthy individuals and patients with pulmonary and cardiac diseases. Myostatin is deeply involved in skeletal muscle repair. In a study of restoration after skeletal muscle injury, myostatin entirely impaired the regeneration process by inhibiting satellite cell activation and myoblast differentiation and transformation (11). Moreover, in most species including humans, myostatin physiologically functions to limit muscle mass. Inhibition of myostatin functions by genetic manipulations as well as congenital anomalies has been known to induce significant muscle growth (12). Therefore, myostatin has been identified as a novel therapeutical target, and several therapeutic approaches, including pharmacological compounds, to inhibit the signal transduction pathway linking to myostatin have been advocated.

4.1.2. Activins and inhibins

Activins and inhibins have been identified as hormonal factors able to regulate follicle-stimulating hormone (FSH) secretion from the anterior pituitary. Two types of inhibins (inhibin A and B) are constituted with a heterodimer of α- and either of two types of β-subunits. By contrast the two β-subunits form three types of dimers, two homodimers and one heterodimer, known as “activins” (activin A, B, and AB, respectively). Both factors belong to the TGF-β superfamily, called the activin/inhibin subfamily, which is different from the DVR subfamily. Activin and inhibin are both known to deeply participate in human reproduction, and their blood concentration changes have been proposed as biomarkers for detecting ectopic pregnancy (13). Therefore, an enzyme-linked immunosorbent assay (ELISA) system able to quantify serum concentration is available for both types of molecule. Activins and inhibins are reported to exert opposing effects in the pituitary for FSH production as shown in Fig. 1.

As shown in Fig. 1, activin forms a complex with type II (ACVR2 and ACVR2B) activin receptors and myostatin, also binding to these same activin receptors for its signal transduction. Therefore, activin is known to negatively regulate muscle growth similarly to myostatin. Ligands that share type II activin receptors are reported to regulate muscle size, and thus activin receptors are noted as therapeutic targets for regulating muscle growth and neuromuscular disorders (14).

Inhibins are also known to act as local regulators leading to cell growth and differentiation. The precise mechanism of inhibin’s action in the skeletal muscle is not yet well characterized. However, as shown in Table 1, results from our muscle-contraction simulation experiments demonstrated a significant increase in mRNA expression of inhibin α-subunits in skeletal muscle. The functional role of inhibin as a modulator of activin signal transduction has been demonstrated (15).

4.1.3. Follistatin

Follistatin was identified as a glycoprotein that inhibits the synthesis and secretion of the follicle-stimulating hormone from the pituitary gland. A highly conserved homology in mammalian species has been reported. Follistatin is not a member of the TGF-β superfamily. However, previous studies on physiological function have demonstrated the intimate relationship between follistatin and muscle growth and hypertrophy. The fundamental role of follistatin as a negative regulator of both activin and myostatin is known (Fig. 1). Their interrelationships are called the activin–myostatin–follistatin (AMF) system, and methods to identify the serum concentration of follistatin in the circulation...
have accordingly been developed (16). Alteration in the follistatin expression or circulating concentration by exercise remains controversial. However, regulation of follistatin’s effect against myostatin and activin are of interest as a therapeutic target for skeletal muscle disorders as well as obesity and energy metabolism.

Follistatin-like protein 1 (Fstl1), also known as TSC36, is reported to have limited similarity with follistatin; therefore, it has been considered a member of the follistatin family. The precise function of Fstl1 is poorly understood, but the circulating Fstl1 level in serum has been reported to increase by exercise training (17).

4.1.4. Irisin

Recently, the proteolytic cleaved extracellular part of fibronectin type III domain–containing protein (FNDC) 5 was named “irisin”. This cleaved fragment, irisin, is now known to be secreted into the circulation in humans, and it is possible to quantify its serum concentration by ELISA (18). The physiological function of irisin is thought to be mediated by the inhibition of myostatin and activin (Fig. 1). Irisin expression has been shown to depend on peroxisome proliferator–activated receptor-gamma coactivator (PGC-1α). It is anticipated for utilization in therapeutic strategies in response to multiple pathological conditions including insulin resistance, lipid metabolism, and chronic kidney disease; and its therapeutic potential has now extended to the central nervous system. Irisin mRNA expression was reported to increase in skeletal muscle by exercise training. However, a randomized controlled training trial failed to show a significant change in irisin serum concentration by training. Moreover, additional experiments have suggested that neither FNDC5 nor irisin is effectively expressed in humans because of the presence of a mutation in the conserved start codon (19).

4.1.4. Bone morphogenic proteins

Bone morphogenic protein (BMP) 4 belongs to the DVR subfamily of the TGF-β superfamily, the same superfamily as myostatin. BMPs are multifunctional growth factors and those activities are well known to be involved in skeletogenesis and differentiation as well as embryonic development (20). Our experiments demonstrated an increase in Huskme mRNA expressions by about 2.8-fold under pressurized conditions (Table 1). BMP4 expression in cultured myoblasts was reported in another study (21), and effects on skeletal muscle myogenesis and myotube formation as well as healing after muscle injury have been observed (22). The pathophysiological role of BMPs as myokines remains largely unidentified and requires further examination.

4.2. Interleukins

The role of inflammation in the pathogenesis of various disorders including atherosclerosis, insulin resistance, and cardiovascular diseases has been well documented. These chronic and moderate inflammations, so-called “low-grade systemic inflammations”, are now recognized as the basis of chronic diseases including MetS. Exercise training is known to have health-beneficial effects by mediating systemic anti-inflammatory responses. The molecular mechanisms that underlie exercise-related anti-inflammatory effects have been partially explained by muscle contraction–induced myokines. Among muscle-contraction–induced productions, interleukins (ILs) are one of the most well recognized exercise-related myokines (23).

IL-6 is known to show enhanced mRNA expression after exercise and is secreted by skeletal muscle cells, leading to serum level elevation. Numerous experiments have described the beneficial effects of IL-6 as a myokine in multiple organs including the central nervous system. These supportive reports emphasize the validity of IL-6 regulation by exercise as a therapeutic strategy (24). However, adverse actions of IL-6 have also been proposed (25). Moreover, reduction of mRNA expression in other members of the IL family, IL-1β and IL-11, was observed in our experiment (Table 1). Even though the existence of other ILs besides IL-6 in skeletal muscle has been observed, their physiological functions are largely yet unidentified.

4.3. Myonectin

C1q/TNF-related proteins (CTRPs) constitute a family of 15 highly conserved secreted proteins, and adiponectin is also known as a representative member. Each CTRP has been reported to exert a specific action on various types of organs, and CTRP15, which is now referred to as myonectin (previous studies sometimes designate CTRP5 as myonectin), is reported to be mainly expressed in skeletal muscle (26). Myonectin mRNA expression has been reported to increase after exercise, and secretion by skeletal muscle cells as well as serum level elevation has also been observed by immunobLOTS in rodents (27). Serum concentration change by exercise was suggested by a semi-quantitative method also in humans (28). The precise physiological roles of myonectin in human peripheral organs are still poorly understood.

4.4. BDNF

Neurotrophins are known to regulate the development, differentiation, survival, and function of nervous system components. At present, four groups of neurotrophins have been identified in humans, and brain-derived neurotrophic factor (BDNF) is among them. Spinal neurons
including Schwann cells and fibroblasts have been largely described as a primary source of these neurotrophins (29). Besides affecting the nervous system, skeletal muscle is also reported as a source of BDNF, and an ELISA system is available for detection of its serum concentration in humans (30). Exercise training has been described to affect serum BDNF concentrations; however, the brain itself has also been assumed to contribute to changes in circulating BDNF (31). Regeneration and myogenesis are predicted as the main role of BDNF in skeletal muscle. Additionally, the beneficial effects of BDNF against impaired glucose metabolism as well as the enhancement of fat oxidation have been pointed out (32).

4.5. Other possible factors

The natriuretic peptide (NP) family (atrial NP, B-type NP, and C-type NP) contains a musclin (osteocrin) as the fourth member. Musclin is reported to bind to the NP clearance receptor (NPR-C) and is thought to compete with ANP for receptor binding. Even though the expression of musclin mRNA has been detected in skeletal myocytes, its physiological function as well as its presence in the circulation is still largely unknown (33).

Fibroblast growth factor-21 (FGF21) is known to be expressed in human skeletal muscle. An ELISA system for the detection of FGF21 serum concentration is available, and the response of FGF21 in skeletal muscle may be regulated by insulin followed by activation of the Akt signaling pathway (34).

There is a report about the presence of a non-neural acetylcholine system that suggests the expression of acetylcholine-like compound in skeletal muscle. However, the precise roles of this compound remain largely unidentified (35).

5. Myokines as therapeutic and pharmacologic targets

Among myokines, the TGF-β superfamily members are the most promising pharmacological targets for multiple disorders (36). It has been anticipated that myostatin regulation will be developed as a strategy for the treatment of metabolic disorders (37), and the results from in vivo experiments using knockout animals have suggested a target for therapeutic approach (38). Irisin (39) and BDNF (40) are also notable candidates for pharmacological interventions.

The AMF system has been shown to involved in several muscle disorders (41) and intervention with this system is believed to be of therapeutic benefit in combating muscle degeneration (42, 43). A recent in vitro study has pointed out the close interaction between skeletal muscle myokines and bone marrow–derived cell function (44). Maintenance of skeletal muscle health status has been shown to have an influence on systemic age-related disorders (45).

Special attention is currently being given to neurotrophines as a therapeutic target for psychiatric and neurological disorders. BDNF is reported to contribute to the reduction of post-ischemic neuronal damage (46), and BMP4 is expected to play a role as a motor neuron survival factor (47).

Amelioration of skeletal muscle function is an important cause of cardiac dysfunction (48). Myokines may provide clues for understanding the relationship between skeletal muscle and cardiovascular diseases and may be new therapeutic targets for maintenance and recovery from cardiac dysfunction.

An exercise-induced factor belonging to the IL-6 superfamily has been reported to affect cancer cell growth (49). However, under the current understanding, myokine secretion into the circulation is thought to depend on muscle contraction. Therefore, exercise may presently be the best way for patients to receive the clinical benefits of myokines, until researchers are able to develop novel methods to regulate myokine secretion.

6. Conclusion

Together with our previous work, we have documented that skeletal muscle is one of the endocrine organs and that understanding the mechanisms of production and secretion of myokines may generate new pharmacological approaches for treatment and management of clinical disorders. We have also shown that our method simulating muscle contraction is valuable for the in vitro study of myokine secretion.

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