Possibility of small-molecule-based pharmacotherapy for sarcopenia

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Abstract  Muscle mass and strength decline with age. When severe, the loss is called sarcopenia. Sarcopenia is drawing attention worldwide, especially in highly aged societies, as a disease that should be treated. At present, we have limited tools to combat sarcopenia (e.g. resistance training and nutritional intervention), but accumulating knowledge of the molecular and cellular mechanisms of sarcopenia is accelerating the development of pharmacological therapies for sarcopenia. Because sarcopenia is a complicated pathological condition caused and modified by many aging-related factors, such as inactivity, loss of motor neurons, poor nutrition, decline of anabolic hormones, chronic inflammation, oxidative stress, impaired stem cell function, and comorbidity, the proposed target molecules or pathways for pharmacological intervention are diverse. Here we review recent progress in drug development with emphasis on small-molecule compound-based therapies and review the literature to identify new therapeutic targets to prevent, delay, or reverse sarcopenia.

Keywords: sarcopenia, selective androgen receptor modulator, muscle plasticity, muscle regeneration, satellite cells, small-molecule compound

Introduction

Skeletal muscle is the most abundant tissue in humans and makes up approximately half of the whole body. Its crucial role is to maintain the stability and mobility of the body by alternating contraction and relaxation of muscle fibers. Skeletal muscle also contributes to whole-body metabolism1. Skeletal muscle mass and function decline gradually with aging2-4. Sarcopenia” is defined as an “excessive loss” of muscle mass and function in aged persons5-9. Morley et al. estimated the prevalence of sarcopenia at 5-13% of persons over 60 years of age on average, and as high as 50% in persons over the age of 80 years10. As the elderly population increases worldwide, sarcopenia is drawing more attention than ever before, not only as a serious healthcare problem but also as a social problem.

Currently, resistance exercise is the primary intervention to prevent and reverse sarcopenia10-12. Leucine-enriched essential amino acids and vitamin D are also shown to enhance muscle function10-13. At the time of writing, no drugs were approved for treatment of sarcopenia, but a number of promising biologic and small-molecule interventions to rejuvenate skeletal muscle are under development.

In this article, we first review the current status of development of so-called selective androgen receptor modulators (SARMs), which are promising in that they have beneficial androgenic effects on bone and muscle, but hardly stimulate the prostate14. We then review the literature describing the molecules and signaling pathways regulating muscle mass and regeneration in animal models, which provide a number of potential pharmacological targets to attenuate sarcopenia. However, we apologize in advance that our review is not comprehensive and does not encompass all the excellent papers related to therapy for sarcopenia.

Approaches using selective androgen receptor modulators (SARMs)

It is well known that supplementation of androgens such as testosterone increases skeletal muscle mass and strength in humans12,13. It is also well established that these anabolic effects of testosterone are related to its dose and circulating concentrations14,15 and exerted mainly via the nuclear androgen receptor, a member of the nuclear receptor superfamily16. However, treatment with supraphysiologic doses is associated with potential cardiovascular and prostate cancer risks and leads to multiple disorders such as erythrocytosis and leg edema15,19; and, therefore, not acceptable as a long-term therapy.

Selective androgen receptor modulators (SARMs) are synthetic androgen receptor agonists designed to produce anabolic effects selectively in skeletal muscle and bone.
without the dose-limiting adverse effects associated with testosterone\textsuperscript{11,20-22}. Structurally, SARMs can be classified into steroidal and non-steroidal types. Initial efforts to develop steroidal SARMs by modifying the chemical structure of testosterone molecules date back to the 1940s, but explorations of non-steroidal SARMs have recently become mainstream.

**LGD-4033.** LGD-4033 is an oral non-steroidal SARM under development by Ligand Pharmaceuticals. In the phase I clinical trial, healthy young male subjects were randomized to receive 0.1, 0.3 or 1.0 mg LGD-4033 or a placebo once daily for 21 days in order to evaluate the safety, tolerability, and preliminary efficacy of the drug. LGD-4033 was found to be safe, had a favorable pharmacokinetic profile, and increased lean body mass (LBM) dose dependently even during this short period without a change in prostate-specific antigen (PSA)\textsuperscript{23}).

**MK-0773.** A phase IIa randomized, placebo-controlled clinical trial to study the efficacy and safety of MK-0773, a steroidal SARM, in elderly females (170 participants aged ≥65) with sarcopenia has been completed by Merck Sharp & Dohme Corp.\textsuperscript{24,25}). Individuals received either 50 mg MK-0773 b.i.d. or a placebo for six months in combination with vitamin D and protein supplementation. A statistically significant increase in LBM from baseline was observed in the MK-0773 group compared to the placebo. However, the MK-0773-induced increase in LBM did not translate to statistically significant improvements in strength or physical function as measured by stair climbing power, bilateral leg press, a short physical performance battery, or gait speed.

**Enobosarm.** Enobosarm (also known as Ostarine\textsuperscript{®}, GTx-024, or MK-2886), a non-steroidal oral SARM developed by GTx, Inc., is the best characterized clinically, and has been tested in phase I, II, and III trials with promising results in terms of improving lean body mass and measurements of physical function and power\textsuperscript{26}. A proof of concept phase II study was conducted during the early stages of clinical development to evaluate the effects of four doses of enobosarm (0.1, 0.3, 1, and 3 mg once daily for 86 days) as compared to a placebo in 120 healthy elderly men (>60 years of age) and postmenopausal women\textsuperscript{27}. Enobosarm increased LBM and improved physical function at the 3 mg dose with statistical significance compared to the placebo. Improvements in insulin sensitivity were also observed in the same dose group. Enobosarm was also evaluated in a phase II clinical trial of cancer patients with muscle wasting using one of two doses of enobosarm (1 or 3 mg) or a placebo, randomized, in a total 159 patients with non-small cell lung cancer (NSCLC), colorectal cancer, breast cancer, non-Hodgkin’s lymphoma, or chronic lymphocytic leukemia. Following four months of treatment, statistically significant increases in LBM were observed with both doses of enobosarm compared to the baseline. Moreover, both doses of enobosarm resulted in significant improvements in stair climbing power over the baseline. No toxic effect associated with androgens and progestational agents emerged in this study\textsuperscript{28}. Two international, pivotal phase III clinical trials, known as POWER1 and POWER2, were initiated in mid 2011 to evaluate the efficacy and safety of enobosarm in preventing and treating muscle wasting in patients with NSCLC, and the U.S. Food and Drug Administration (FDA) has selected enobosarm for the fast track program. In each of these placebo-controlled, double-blind clinical trials, approximately 325 patients with stage III or IV NSCLC were randomized to oral daily doses of a placebo or 3 mg enobosarm for 147 days when they began first-line standard chemotherapy. Unfortunately, in August 2013, GTx, Inc. announced that the POWER studies had failed to meet the co-primary endpoints of LBM and physical function that were assessed statistically using responder analyses\textsuperscript{29}. In the POWER 1 study, enobosarm had significantly improved LBM in NSCLC patients on first-line chemotherapy with platinum plus taxane after 84 days of once-daily treatment compared with a placebo. However, the improvement was not seen in POWER2, which employed the same study design, but involved NSCLC patients on first-line chemotherapy with platinum plus non-taxane. In this trial, enobosarm was not significantly better at improving LBM than the placebo. Physical functioning, which was the other primary endpoint and was assessed by stair climbing at day 84, was not significantly improved in either trial. Although the current data from the POWER trials were insufficient to support the filing of a new drug application for treatment of muscle wasting associated with advanced NSCLC, these findings are meaningful to the development of drugs for the treatment of sarcopenia.

**Others.** Other than the above clinical candidates, phase I clinical studies of BMS-564929 (Bristol-Myers Squibb\textsuperscript{30-32}), GLPG0492 (Galapagos)\textsuperscript{33,34}, LY2452473 (Eli Lilly)\textsuperscript{35}, GSK971086 (GlaxoSmithKline)\textsuperscript{36}, and S-101479 (Kaken Pharmaceuticals)\textsuperscript{37,38} have been implemented. In addition, a pre-clinical study of castrated rats demonstrated that NEP28 (Sumitomo Chemical) strongly enhanced the development of muscle, and restored muscle weight to the eugonadal level without excessive undesired pharmacological effects on the prostate\textsuperscript{39}.

**IGF1-PI3K-Akt-mTOR pathway**

Maintenance of skeletal muscle mass is regulated by a balance of protein synthesis and degradation\textsuperscript{38,39}. Because the rate of protein breakdown following resistance exercise in muscles of individuals with sarcopenia is the same as that of young controls\textsuperscript{40}, a deficit in protein synthesis might be the basis for age-associated muscle loss.
The insulin-like growth factor-1 (IGF-1)-phosphoinositide 3-kinase (PI3K)-Akt-mammalian target of rapamycin (mTOR) pathway is a major pathway to positively regulate muscle mass. mTOR is also activated by other growth factors, glucose, and amino acids, as well as by physical activity such as exercise and mechanical stress. and, in turn, phosphorylates its downstream targets p70S6K and PHAS-1, which leads to increased protein synthesis and muscle mass.

Activation of the IGF1-PI3K-Akt-mTOR pathway as a means to enhance muscle function has been the focus of intense investigation. In fact, the therapeutic effects of IGF-1 by administering its protein or overexpressing the gene have been reported in dystrophic rodents. Nevertheless, we still know hardly anything about the drugs to modulate the IGF-1/Akt/mTOR pathway, except for NBI-31772. This compound is an IGF-1 aptamer, which binds IGF-binding proteins (IGFBP) and inhibits IGF binding to IFGFB by displacing IGF-1, and, consequently, increases the circulating level of IGF-1 and potentiates its action. Schertzer et al. showed that administration of NBI-31772 enhanced the functional repair of muscle fibers after myotonic injury and ameliorated the pathology of dystrophic mice. Likewise, pharmacological stimulators of protein synthesis hold promise for the treatment of sarcopenia.

**TGF-β signaling pathway as a therapeutic target for sarcopenia**

The transforming growth factor-β (TGF-β) signaling pathway is pivotal in controlling developmental muscle growth. The TGF-β family of ligands that activate this pathway, such as myostatin (GDF8), TGF-β, BMP, or activin, act through type I and type II trans-membrane serine/threonine kinase receptors in the membrane of the muscle cell and effector Smad proteins, predominantly through phosphorylation and nuclear translocation of Smad2 and Smad3 transcription factors, which form heterodimers with Smad4 to regulate target genes involved in muscle growth and wasting.

In 1997, myostatin was first demonstrated to be a potent negative regulator of muscle mass in genetically manipulated myostatin-null mice, and then naturally occurring mutations in the myostatin gene were identified in the heavily muscled Belgium Blue cattle, Whippets, a breed of racing dogs, and a German boy. Myostatin is expressed mainly in skeletal muscle, and to a lesser extent in adipose tissue and cardiac muscle. Although specific transcriptional targets remain unclear, it is widely accepted that myostatin, via activation of Smad2 and Smad3, promoted muscle atrophy both by inhibiting the Akt/mTOR/p70S6K pathway and by inhibiting gene expression of MyoD or myogenin, transcription factors that play crucial roles in satellite cell differentiation. Inhibition of myostatin signaling by genetic means or antibodies caused dramatic increases in muscle mass. Although it is not clear whether or not the abundance or activity of myostatin is affected by aging, myostatin inhibition is expected to be a promising therapeutic approach for the prevention of muscle wasting in aged persons.

In attempts to modulate the TGF-β signaling pathway, the strategies using biologics (neutralizing antibodies, propeptides, soluble ActRIIB receptors, and interacting proteins, such as GASP-1, follistatin, and FLRG) are currently mainline. For example, LY2495655, a humanized antibody designed to bind to myostatin, was evaluated in a phase II clinical trial by Eli Lilly of cancer patients with muscle wasting (Clinicaltrials.gov identifier: NCT01505530). ACE-031, a soluble form of activin receptor type IIB, has been developed by Acceleron Pharma/Shire. ACE-031 showed a strong capacity to decrease body fat, increase muscle mass, and improve insulin sensitivity, but safety concerns were raised in a phase II clinical trial (NCT01099761). Recently, Acceleron Pharma announced that it has initiated a phase I clinical trial of ACE-083, a novel protein therapeutic that acts as a ligand trap for members of the TGF-β superfamily (NCT02257489).

Other alternatives to manipulate the myostatin pathway include the delivery of pro-peptides that can bind to and block myostatin in solution or by transient DNA expression, and elicit muscle growth and improve muscle function. For long-term treatment of older persons with sarcopenia, an intervention selectively targeting myostatin without affecting other TGF-β family members, such as activin A signaling, would be required.

**Restoring satellite cell function**

Skeletal muscles possess great ability to regenerate, but this ability becomes compromised as people age. Hence, restoration of the regenerative activities of aged skeletal muscle is expected to be a therapeutic option for sarcopenia.

Regeneration of skeletal muscle is essentially sustained by resident muscle stem cells, satellite cells (SCs), located between the basal lamina and the sarcolemma of myofibers in adult muscle. When muscle is damaged, quiescent SCs are activated, vigorously proliferate at the site of the injury, and form new multinucleated myofibers or fuse with partially injured muscle fibers. At the same time, a small fraction undergoes self-renewal to maintain the stem cell pool for future requirements. Several studies have identified small molecules that promote muscle regeneration by activating SCs.

**SIP promotion of muscle regeneration**

Sphingosine-1-phosphate (S1P), which is a bioactive sphingolipid known to control cell growth, is widely recognized as a key player in skeletal muscle regeneration.
In 2006, Nagata et al. first reported that S1P is involved in the entry of dormant SCs into the cell cycle when muscle is injured\(^{71}\). The authors also demonstrated that the inhibition of S1P synthesis perturbed muscle regeneration\(^{71}\). Danieli-Betto et al. demonstrated that quiescent SCs express several kinds of S1P-specific receptors, and that direct injection of S1P into myotoxically injured muscle significantly increased the diameters of regenerated fibers in rodent experiments\(^{76}\), suggesting that S1P signaling promotes the regenerative processes of skeletal muscle. Another study by Ieronimakis et al. showed that increased S1P through direct injection or via the administration of the small molecule 2-acetyl-4(5)-tetrahydroxybutyl imidazole (THI)\(^{75,76}\), an inhibitor of S1P lyase, has beneficial effects in acutely injured dystrophic mice. Actually, the mice treated with THI had a fourfold increase in the number of cells expressing Myf5, a key regulator in myogenesis. More importantly, THI-treated mice showed a 3.6-fold increase in the number of newly regenerated myofibers, or STAT3 inhibitor (5, 15 DPP) into CTX-injured muscle significantly increased the diameters of regenerated fibers of old and young (3-month-old) adult mice. Compared with vehicle-treated regenerated myofibers, pharmacologically treated regenerated myofibers in both the old and young adult mice were wider and had more SCs, fewer infiltrated macrophages, and better architecture. Moreover, leg muscles treated once three days after CTX injury with either of the inhibitors were stronger and slower to fatigue, implying that JAK-STAT inhibition promotes functional recovery of muscle\(^{80}\).

**Angiotensin II type 2 receptor signaling in SCs**

An additional interesting target associated with muscle regeneration, that has been recently reported by Yoshida et al., is angiotensin II (Ang II) type 2 receptor (AT2R) signaling\(^{79}\). Patients with advanced congestive heart failure or chronic kidney disease often have increased Ang II levels and symptoms of cachexia\(^{83-85}\). Ang II infusion in rodents causes sustained skeletal muscle wasting and symptoms of cachexia\(^{79-82}\). Ang II infusion or chronic kidney disease often have increased Ang II signaling positively regulates myoblast differentiation and potentiates skeletal muscle regeneration, providing a new therapeutic target in muscle-wasting disorders.

**JAK-STAT signaling in SCs**

Just recently, two research groups reported that inhibition of Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling can restore the function of SCs in aged and dystrophic muscle.

Price et al.\(^{80}\) first performed genome-wide expression analyses and identified significantly higher expression of JAK-STAT signaling targets in SCs from 18-month-old mice relative to those in 3-week-old mice. Next, SCs treated in culture with the JAK2 inhibitor tyrphostin AG490 (Tyr AG490)\(^{80}\), the STAT3 inhibitor 5, 15-diphenylporphine (5, 15-DPP)\(^{80}\), or siRNAs targeting STAT3 or JAK2 underwent markedly more symmetric cell divisions than control SCs, suggesting that increased STAT signaling in older SCs may prevent SC self-renewal. Finally, the authors injected a JAK2 inhibitor (Tyr AG 490) or STAT3 inhibitor (5, 15 DPP) into CTX-injured muscle of old and young (3-month-old) adult mice. Compared with vehicle-treated regenerated myofibers, pharmacologically treated regenerated myofibers in both the old and young adult mice were wider and had more SCs, fewer infiltrated macrophages, and better architecture. Moreover, leg muscles treated once three days after CTX injury with either of the inhibitors were stronger and slower to fatigue, implying that JAK-STAT inhibition promotes functional recovery of muscle\(^{80}\).

Tierney et al.\(^{81}\) was motivated by previous findings suggesting that elevated levels of inflammatory cytokines such as interleukin-6 (IL-6) and their downstream effector STAT3 are associated with both age-related and muscle-wasting conditions\(^{82-90}\). They revealed that IL-6-activated STAT3 signaling regulates satellite cell behavior, promoting myogenic lineage progression through MyoD regulation. In Pax7-CreER; Stat3\(^{lox/lox}\) mice\(^{97,98}\) in which STAT3 was conditionally knocked out of SCs, muscle injury by notexin (NTX) triggered an expansion of SCs, and myofibers were repaired more rapidly; however, the diameters of the regenerated myofibers at 25 days after injury were smaller than those in wild type controls. Therefore, although ablation of STAT3 signaling enhances SC expansion, it also compromises muscle repair. The authors next investigated whether pharmacological inhibition of STAT3 could promote SC expansion, yet allow proper regeneration of muscle fibers. Indeed, intermittent intramuscular injection of a STAT3 inhibitor accelerated muscle regeneration in NTX-injured muscles of aged (24-month-old) mice as well as in dystrophic mice, and gave rise to larger regenerated myofibers after four weeks compared with vehicle-treated animals. Finally, the effects of STAT3 inhibition on cell fate and proliferation were conserved in human myoblasts\(^{91}\). Together, these results suggest that pharmacological manipulation of JAK-STAT signaling activity can counteract the functional exhaustion of SCs in muscle-wasting conditions, thereby maintaining the endogenous regenerative response and
Importantly, inhibitors of JAK-STAT3 signaling, such as ruxolitinib (JAK1 and JAK2 inhibitors) and tofacitinib (JAK3 inhibitor), have already been approved for treatment of cancer and inflammatory disorders including psoriasis, myelofibrosis, and rheumatoid arthritis. Additional JAK-STAT inhibitors are in late-stage clinical trials for an even broader range of diseases. Regeneration of diseased or injured muscle may represent another indication for such compounds.

Enhancement of self-renewal of aged SCs by inhibiting p38 signaling pathway

Bernet et al. and Cosgrove et al. reported that aged SCs are characterized by elevated p38 mitogen-activated protein kinase (MAPK) signaling, which inhibits self-renewal of aged SCs, and increases the number of myoblasts committed to terminal differentiation. The authors assert that these properties of aged SCs explain why muscle regeneration in aged mice is insufficient. Although the studies have been performed on only 2-month-old (young) and 24-month-old (aged) mice so far, pharmacological inhibition of the p38 MAPK cascade is also an attractive therapeutic strategy for treatment of sarcopenia in aged humans.

Circulating factors in young blood for rejuvenating skeletal muscle

Using heterochronic parabiosis experiments, Rando and his colleagues showed that the functions of aged SCs were restored in younger environments. More recently Sinha et al. demonstrated that GDF11 is a major humoral factor that rejuvenates old SCs. Interestingly, recombinant GDF11 directly stimulated proliferation and differentiation of aged SCs in vitro. Thus, stimulating the GDF11 signaling pathway is another possible avenue to attenuate age-related loss of muscle mass and function. In a separate paper, Sinha et al. also reported that testosterone contributes to enhanced skeletal muscle growth in aged mice using heterochronic parabiosis between young and old mice.

Is impaired function of aged SCs really a major cause of sarcopenia?

During the writing of this manuscript, Fry et al. reported that inducible depletion of muscle SCs by genetic manipulation in adult mice impaired muscle regeneration, but did not exaggerate sarcopenia. This study questions the rationales of satellite cell–centered therapeutic interventions for sarcopenia. In the muscular dystrophy field, impaired function of SCs and reduced regenerative capac-
ity are undoubtedly targets of therapy, but it would be inappropriate to directly translate the findings obtained with muscular dystrophy models to the therapeutic strategy for sarcopenia.

**Main issues to be overcome in developing drugs for treatment of sarcopenia**

**Avoiding adverse effects of small-molecule therapeutics.**

Although many strategies for treatment of sarcopenia may be promising, it is important to point out the major concerns associated with small-molecule therapeutics. One is their nonspecific adverse effects\(^\text{(108,109)}\). Due to their small size, small molecules can easily access non-target cells and elicit unwanted physiological responses\(^\text{(110)}\). In the case of MK-0773, one of the aforementioned SARMs, suspension of further development will probably be inevitable because liver transaminases elevations were observed in a greater number of participants of the MK-0773 group than the placebo group in a phase IIa clinical study\(^\text{(24)}\). In the case of ACE-031, a chimera of activin receptor type IB also mentioned above, although safety concerns that preclude further development have not officially been published, it is presumed that this molecule has off-target effects, given its lack of target tissue specificity.

Two main approaches to overcome these issues could be cited generally. It goes without saying that the first approach is modifying the chemical structure of compounds to achieve high selectivity for target molecules. The second is targeting a molecule that has high specificity for skeletal muscle tissue. We may also refer to effective delivery strategies as a third unconventional approach. The possibility of adopting methods of local administration, such as intramuscular injection, should be considered in order to avoid the problem of systemic side effects, even if oral treatment is indeed the most convenient for sarcopenia patients. In this case, precise and spatial control of drug dosage in target muscle tissue may be enabled by using sustained-release techniques or advanced materials such as hydrogel with compounds.

**Overcoming regulatory hurdles for drug development.**

Another issue in the development of drugs for treatment of sarcopenia is the regulatory hurdles. While the regulatory pathway for the approval of drugs for other diseases has been well delineated because of precedence set by previously approved drugs, the road for approval of sarcopenia therapies has not been clearly established. In order to design the proper protocol for clinical studies and help clinical assessment of patients as well as recruitment into trials, it is primarily important to set the appropriate inclusion and exclusion criteria for enrolling patients. Sarcopenia is a syndrome characterized by aging-related loss of skeletal muscle mass and function\(^\text{(7,9)}\), but different definitions of and criteria for treatment of sarcopenia have been proposed by several international academic groups\(^\text{(111-114)}\). Increasing the effort to generate consensus around efficacy of outcomes in pivotal trials and establish minimal clinically important differences in key efficacy outcomes will facilitate the clinical trials of candidate molecules.

**Conclusion**

Sarcopenia, the aging-related decline in skeletal muscle mass and function, causes loss of independence in older people. Therefore, it has become a major focus for drug discovery in aged societies. Although no medicine has been approved by international regulatory agencies for sarcopenia (as of the end of 2014), many therapeutic approaches for sarcopenia, especially pharmacological intervention with small-molecule compounds, have been reported. The authors believe that a drug discovery for sarcopenia will be accelerated by continuing efforts to clarify the mechanisms of aging-related muscle loss and dysfunction, and by the collaboration between academic scientists, physicians, and pharmaceutical companies.

**Conflict of Interests**

The authors declare that the manuscript was written in the absence of any commercial or financial relationships, although Yuka Watanabe is a research fellow employed by Astellas Pharma Inc.

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