をシナプス襞に結合するアンカー蛋白として必要であり，コリン過敏性の原因はこの機能を抑制するためにおきる可能性が考えられる[20][31]．

おわりに

我々は，神経筋シナプスを介した運動神経と筋の相互維持作用による維持システムを知るために分子，細胞，動物レベルのイメージング技術による革新的な研究方法の開発を行っている．マウスなどの疾患動物モデルとシビレエイの電気器官（神経筋シナプスが巨大化した組織）を使った基盤研究の成果を，ヒト対象としたサルコペニアと筋萎縮性神経筋疾患を対象とした医療へ展開したいと考えている．完全に萎縮した筋の回復は現在も不可能ですから，医療現場ではイノベーションが強く求められている．急速に展開する幹細胞・iPS細胞技術を用いた移植医療に期待がよせられている．しかし，萎縮した筋の再建には細胞補充療法だけでなく筋・神経維持システムの理解に基づく再構築が必要である．筋は運動神経の従属的な支配下にあるのではなく，むしろ筋が運動神経を維持する分子機構があるからだ．我が国で神経筋シナプスに興味を持って研究するグループはほとんどない．シナプスの研究は運動神経や筋を対象とした新しい概念に基づく研究分野へと発展させたい．筋と運動神経を含む運動器の健康維持に関わる医療技術の開発には，シナプス研究の成果を取り入れる必要がある．

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特集
転倒危険者の早期発見から予防まで
―最新のエビデンスから―
Topics

KEY WORD
●サルコペニア ●加齢性筋肉減少症
●介護予防 ●サテライト細胞

2．サルコペニア(加齢性筋肉減少症)とそのバイオマーカー

重本 和宏
丸山 直記

SUMMARY

■サルコペニア(加齢性筋肉減少症)は，認知症とともに高齢者のADLとQOLを損なう主要な原因である．早期発見とリハビリの効果を判定する客観的な指標は介護予防対策に必要であり，サルコペニアの病態メカニズムの解明と併せてバイオマーカーの開発が求められる．

はじめに

筋力測定や日常生活の運動能力のスコアによる判定は，筋萎縮を既に伴うような筋力低下を検出することは容易であるが，早期発見と予防に対して有効とはいえない．サルコペニアの原因は以下の3種類に分類することができる．まず，筋と運動神経細胞を取り巻く体内環境全体の変化(ホルモン，炎症や筋分化因子の変化)による老化，幹細胞(サテライト細胞)とそれを維持する微少環境(ニッチ)の老化，さらに筋と運動神経細胞(中枢神経)の相互作用による維持システムの老化であり，これらの相互作用で病態を進行させると考えられる(図)．

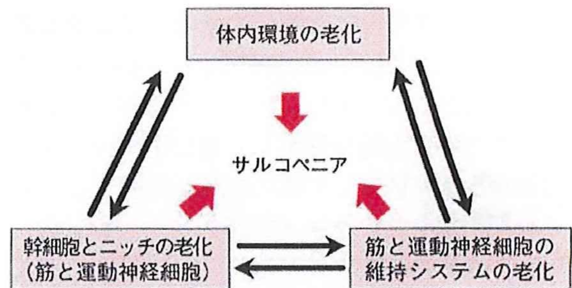

図 サルコペニアの原因
遺伝因子，環境要因，エピジェネティックの変化で誘発される3種類の経路．

体内環境の変化によるサルコペニア

加齢に伴い，慢性の分子的炎症が生じて老化現象が顕在化する可能性が提案されている．分子炎症の指標としてIL-6，CRPやα_1-antichymotrypsinなどがある．Shaapらは，上記の分子指標とサルコペニアに関連を検討した．その結果，血中IL-6量は握力と四肢の筋量と逆相関を示した．高IL-6(＞5 pg/mL)や高CRP(＞6.1 μg/mL)は40%以上の筋力低下の危険率が2～3倍となる．また筋量の低下は少ない．高α_1-antichymotrypsin量は筋量のわずかな減少と相関する．加齢に伴う血中慢性炎症のマーカー量の増加は，その種類によっては筋力低下の程度と相関するが，筋量とは必ずしも相関しないと報告された．慢性炎症性マーカーがどのようなメカニズムで筋力低下に関与するかは，今後の解析が必要となる．しかし動物実験では，必ずしもヒトで認められた現象は追認されていないことも報告されている[1]．

疫学的調査により腎機能の低下がサルコペニアの出現と相関することが報告されている．Foleyら[2]はサルコペニア発症とGFR低下の相関を報告している．ほかの要素として低所得，肥満，運動不足，栄養の偏り，高カルシウム血症，ビタミンDの低摂取，高拡張期血圧およびインスリン抵抗性がサルコペニアと関連すると報告された[2]．加齢に伴い筋量が減少し，一方で脂肪量が増加するが，これは「sarcopenic obes-

■しげもと かずひろ(地方独立行政法人 東京都健康長寿医療センター研究所研究部長)／まるやま なおき(地方独立行政法人 東京都健康長寿医療センター研究所副所長)

ity」と呼ばれて注目されている[3]．脂肪組織，特に内臓脂肪組織から炎症性サイトカインが分泌され，筋肉組織での異化作用が亢進しsarcopenic obesityを誘導すると考えられている．Schragerらの疫学的解析から，高IL-6，CRP，IL-1受容体拮抗分子，可溶性IL-6受容体などが相関するとされている[4]．炎症性サイトカインがサルコペニアを誘導する機序は今後の解析を必要とするが，サルコペニアのバイオマーカーとして利用できる可能性がある．

高齢マウスを使った実験から，体内環境でサテライト細胞の機能低下と筋の線維化を促進する因子としてWntが報告された[5]．高齢マウスと若いマウスの2匹の血管を縫合して血液循環を同じにして(parabiotic pairings)，筋の再生能を調べたところ，若いマウスとつながった高齢マウスの筋再生能は増強し，逆に若年マウスでは筋再生能が低下して筋の線維化が進んだ．その促進因子としてWntを同定した[5]．Wntは，肺や肝臓の線維化の促進因子としても報告されている．さらにKlothoはWntと結合することで細胞老化を抑制するらしい[6]．Wntとその抑制因子はサルコペニアのバイオマーカーとなる可能性があり，ヒトにも応用できるかどうか今後の研究が注目される．

サテライト細胞と微少環境の老化によるサルコペニア

高齢による筋の再生能の低下は，サテライト細胞の微少環境の老化と関係するという報告がある．サテライト細胞の微少環境として，周囲の筋細胞や細胞外マトリックスなどがある．高齢マウスのサテライト細胞を若いマウスの筋に移植すると十分な再生能を示す．一方Conboyらは，若いマウスのサテライト細胞を高齢マウス由来の筋と共培養すると，再生能が顕著に抑制することを示した[7]．高齢マウスの筋の基底膜にはTGF-βが増加していた．TGF-βはサテライト細胞の増殖を抑制した．またサテライト細胞の増殖刺激に必要なNotchシグナルは，逆に減少していた[8]．前に紹介した体内環境と微少環境(ニッチ)との関係は不明であるが，TGF-βのシグナルに関係する因子とサルコペニアの因果関係が注目される．

運動神経と筋の相互維持システムに着目した筋萎縮の早期バイオマーカーの探索

もともと健常筋には萎縮へと向かうカスケードが常在しているが，適切な運動習慣により，運動神経の終末と筋のつなぎ目である神経筋シナプスを介した筋と運動神経の相互作用が，萎縮カスケードに拮抗することで筋と運動神経の両方が保持する．これは，運動神経と筋のいずれかの原因によってこの維持メカニズムが阻害されると筋萎縮が進むことを示す．われわれは，この相互維持を担う分子は筋萎縮の早期発見のバイオマーカーになると考え研究を進めている．筋から産生されて運動神経を維持する因子は，その有力な候補である．運動神経と筋の維持メカニズムに立脚したバイオマーカーの発見は介護予防の指標となるだけでなく，筋萎縮性疾患の革新的な治療法の開発にもつながるであろう．

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The Immunopathogenesis of Experimental Autoimmune Myasthenia Gravis Induced by Autoantibodies against Muscle-specific kinase (MuSK EAMG)

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Introduction

Myasthenia gravis (MG) is the most common disorder of neuromuscular synapses and well-recognized for such characteristic clinical features as ptosis, fatigue and muscular weakness. Ptosis and diplopia occur early in most of these patients. With passing time, when the weakness of bulbar and respiratory muscles worsens, the disease becomes life-threatening so that intubation with mechanical ventilation is required. In 1905, Buzzard postulated that a circulating “autotoxic agent” causes the muscle weakness as lymphocytes infiltrate muscles and tumors of the thymus gland. He also believed that such muscle degeneration was closely related to Graves disease and Addison disease, both of which are now regarded as autoimmune diseases. In 1960, Simpson and, independently, Nastuk et al. proposed that MG is caused by an autoimmune mechanism.

In 1973, seminal studies done by Patrick and Lindstrom first demonstrated that autoantibodies against nicotinic acetylcholine receptors (AChRs) at neuromuscular junctions (NMJs) cause MG. This conclusion came from their work with an animal model they called experimental autoimmune myasthenia gravis (EAMG) (1). To develop this model, they inoculated rabbits with AChR protein purified from electric eels and showed that the resulting AChR antibodies induced muscle weakness and paralysis. In fact, these antibodies produced in response to the eel AChR protein cross-reacted with rabbit AChRs at the NMJs. The flaccid paralysis and results from electrophysiological studies of animals with EAMG closely resembled manifestations in patients with MG. In 1976, Lindstrom used the newly developed immunoprecipitation assay of that sera with

radio-labeled human AChRs and discovered that approximately 80% of patients with MG had serum antibodies to AChRs (2). Further, histological and functional studies of NMJs in both MG patients and animals with AChR EAMG demonstrated a loss of AChR numbers and function and failure of neuromuscular transmission leading to muscle weakness (3, 4). However, ~20% of MG patients' autoantigens were never identified, although accumulating evidence showed the existence of autoantibodies against NMJs. MG patients bearing the latter autoantibodies responded well to plasma exchange and immunosuppressive therapies (5). Additionally, passive transfer of plasma immunoglobulin from such MG patients into mice caused a failure of neuromuscular transmission as shown by electrophysiological studies (6).

In 2001, Hoch and Vincent found autoantibodies against muscle-specific kinase (MuSK) in 70% of generalized MG patients who lacked antibodies to AChRs and demonstrated that MuSK antibodies in the sera inhibited MuSK functions in culture myotubes (7). MuSK is indispensable for neuromuscular development, as proven when MuSK knockout mice failed to form AChR clusters or differentiate postsynaptic regions and, therefore, died perinatally (8). Considering its performance of such functions in developing NMJs, MuSK seemed to be the long-sought autoantigen in MG patients; nevertheless, the pathogenic role of anti-MuSK antibodies has been unclear. First, no experimental animal model of MG has been induced by MuSK (9). Second, passive transfer of MuSK antisera from MG patients does not generate the equivalent disease in mice. Apparently the pathogenicity of anti-MuSK antibodies still requires proof by establishing an EAMG like that incited by AChRs (9).

In 2006, for the first time, the inoculation of rabbits with purified MuSK protein caused a loss of AChRs and muscular weakness by disrupting neuromuscular transmission (10). Next, MuSK EAMG was also successfully established in mice (11, 12). In 2008, passive transfer of a large amount of MuSK antibodies from MG patients into mice caused EAMG (13). Therefore, the pathogenicity of MuSK antibodies has now been proven by inciting MG experimentally via active immunization with MuSK and also by passive transfer of MuSK antibodies from patients with MG.

However, if we turn our attention to the clinical studies of MG patients with anti-MuSK antibodies (termed MuSK-MG patients), several complex issues remain unresolved. First, no significant loss of AChRs at NMJs was observed in biopsies from biceps brachii muscles of MuSK-MG patients (14). Second, MuSK antibodies are mainly in the IgG4 subclass, which does not activate complement (15-17), yet complement-mediated damage to postsynaptic membranes is considered a major source of pathogenicity in MG patients with AChR antibodies. Third, a number of clinical studies have shown that MuSK MG constitutes a distinct subclass of the disease (18-20). MuSK-MG patients more often develop severe muscle weakness and eventual atrophy than AChR-MG patients, and the former respond differently to therapy than persons in the latter group. Fourth, although antibodies to AChR in MuSK-MG sera have barely been detected using routine radio-immunoprecipitation assays, low affinity IgG and IgM antibodies to AChR were detected with an immunofluorescent technique in some MuSK-MG patients by using human embryonic kidney cells expressing recombinant AChR subunits on the cell surface (21). Studies of MuSK EAMG will contribute to

understanding both the pathogenicity of anti-MuSK antibodies and the clinical features of MG patients.

In this chapter, we will first describe the EAMG caused by anti-MuSK antibodies and discuss the possible pathogenic roles of the antibodies associated with the clinical features of MuSK-MG patients. Finally, we suggest the wisdom of using animals with MuSK EAMG for the development of more effective medication.

1. Induction of MuSK EAMG in animals

The first piece of evidence that active immunization with MuSK protein induced MG-like muscle weakness in animals came from experiments with rabbits (10). The muscle weakness of such rabbits resembled that observed when purified AChR was first used to induce EAMG in rabbits (1). After anti-MuSK antibodies were generated by inoculating rabbits with recombinant soluble MuSK protein, the recipients developed flaccid paralysis. Even before Hoch and Vincent reported the existence of anti-MuSK antibodies in the sera of generalized MG patients (7), the ability of these antibodies to inhibit MuSK functions had been serendipitiously studied. Recombinant proteins from the extracellular portion of mouse MuSK were generated as antigens. The extracellular segment of MuSK comprised five distinct domains, i.e., four immunoglobulin-like domains and one cysteine-rich region. The fusion protein expression construct, which consisted of the mouse MuSK ectodomain with His-tag, was generated and transfected into COS-7 cells (Figure 1). The secreted recombinant MuSK-His proteins were purified by using histidine affinity columns. New Zealand White rabbits were then immunized

with 100 to 400 μg of the purified MuSK recombinant protein. After three to four injections of MuSK

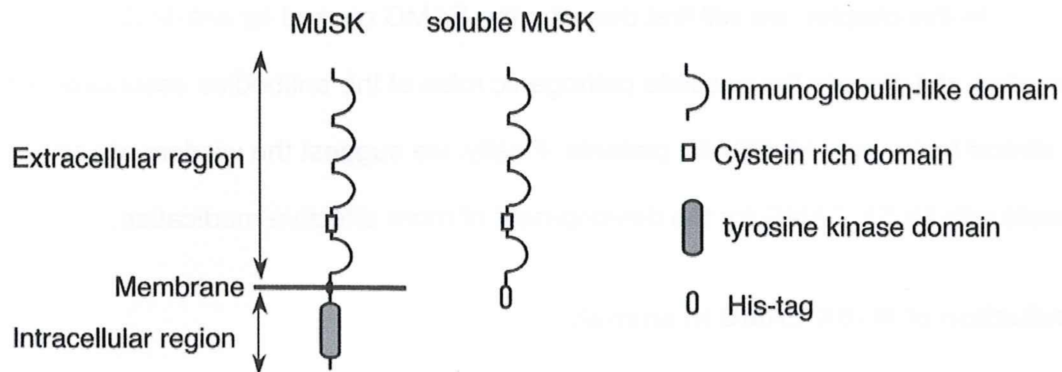

Figure 1. Schematic representation of MuSK proteins. Soluble recombinant protein of MuSK contains His-tag at the C-terminal end.

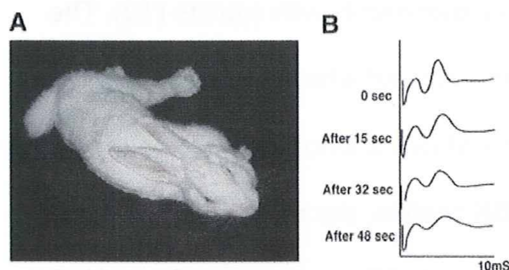

Figure 2. A rabbit manifested MG-like paresis after immunization with soluble MuSK protein (A). Electromyograms of the paretic rabbit show a decline in compound muscle action potential (CMAP) during repetitive nerve stimulation. (Reprinted with permission from Shigemoto et al., J Clin Invest 116:1016-1024. 2006)

protein, all of six rabbits manifested flaccid paralysis (Figure 2A). Sera from the paretic rabbits contained a high titer of anti-MuSK antibodies that reacted specifically with MuSK molecules on the surfaces of C2C12 myotubes as observed in sera from MG patients who were positive for anti-MuSK antibodies (7, 10). In repetitive electromyograms from one of these paretic rabbits, the retroauricular branch of the facial nerve was stimulated at 20 Hz, and recordings were taken from adjacent retroauricular muscle. The compound muscle action potential (CMAP) showed a decremental pattern, consistent with the typical MG (Figure 2B). However, injections of acetylcholinesterase inhibitor (Neostigmine) did not significantly reverse either the CMAP defect or the paralytic symptoms in the rabbit EAMG.

Inoculation of MuSK protein also successfully caused MG-like weakness in mice (11, 12). In certain mouse strains, these injections with a recombinant rat-MuSK extracellular domain elicited clinical signs resembling those seen in MG patients. Additionally, the muscle weakness of MuSK-injected mice was similar to that observed in rabbits injected with purified AChR or MuSK (1, 10) and was accompanied by electrophysiological changes that resembled those of MG (Figure 3A). Injections of Neostigmine only partially reversed the CMAP defect (Figure 3B).

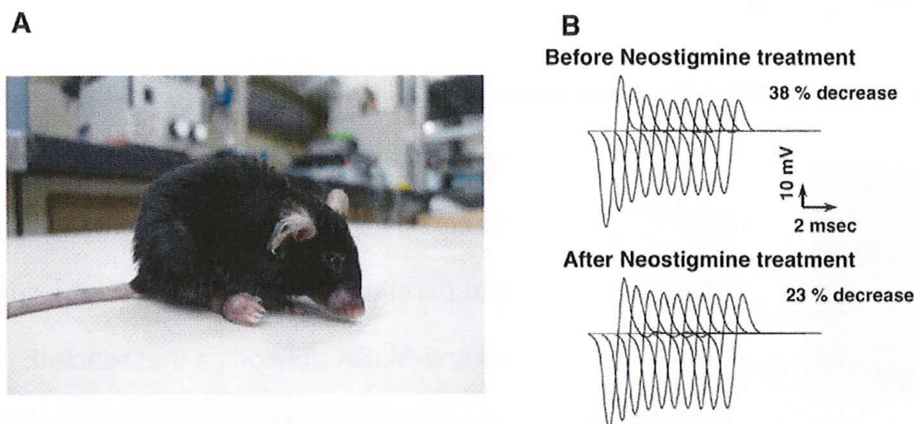

Figure 3. Injection of soluble MuSK protein into mice induces MG-like muscle weakness (A). Electromyograms of the paretic mice show a decline in CMAP during repetitive nerve stimulation (B). Treatment of acetylcholine-esterase inhibitor (Neostigmine) can only partially improve the decremental response to repetitive nerve stimulation.

To assess susceptibility to muscle weakness caused by MuSK immunization in mice, four different strains of mice were injected with the same dose of MuSK proteins (11). Mice of the following strains: C57/BL6 (B6, n =25), A/J (n =15), B6.C-H-2 ^{bm12} (bm12, n =10) and BALB/c (n =5) were injected on days 0 and 28 with MuSK (10 μg/mouse) followed by a third injection at day 56 for mice without severe disease (23 B6, 6 A/J, 10 bm12 and 5 BALB/c mice). Muscle weakness was evaluated by an exercise test (Table 1). In three strains, B6, A/J and bm12, the majority of MuSK-injected mice

responded with at least mild signs of fatigable muscle weakness after the second injection and severe signs of weakness after the third injection (Table 1).

Strains	No.of injections	No. of mice	Muscle weakness MG grade 0	1	2	3	% mice showing EAMG	Average grade of all mice
B6 ($H2^b$)	2	25	8	6	8	3	68	1.24
	3	23	3	4	7	9	87	1.96
A/J ($H2^a$)	2	15	1	1	3	10	93	2.47
	3	6	0	1	1	4	100	2.50
bm12($H2^{bm12}$)	2	10	2	4	4	0	80	1.20
	3	10	2	2	3	3	80	1.70
BALB/c ($H\text{-}2^d$)	2	5	5	0	0	0	0	0.00
	3	5	5	0	0	0	0	0.00

Table 1. Quantitative assessment of muscle weakness in four mouse strains after the second and third injections of MuSK. Mice received injections of MuSK on days 0, 28 and 58. Grades for muscular strength were as follows: Grade 0, no weakness after exercise test consisting of 20 consecutive paw grips on cage-top steel grids; Grade 1 (moderately decreased activity), mild muscle fatigue after exercise; Grade 2 (markedly decreased activity), hunched posture at rest; Grade 3 (severe generalized weakness), loss of weight and inability to ambulate. (Reprinted from *J Neuroimmunol*. Jha et al., 175:107-117.2006 with permission from Elsevier.)

Susceptibility to MG clearly differed among mouse strains. B6 and bm12 mice were highly susceptible, which coincided with their higher anti-MuSK-antibody responses. However, A/J mice were even more susceptible, and the most severely affected had lower titers of anti-MuSK antibodies compared to B6 and bm12 strains, which were clinically less affected (Table 1, Figure 4). The B6 and bm12 strains differ by only 3 amino acids in the β chain of the I-A subregion. We must note that A/J is one of the strains that develop a late onset (four to five months) progressive muscular dystrophy as a result of a homozygous retrotransposon insertion in the dysferlin (*Dysf*)

gene, and phenotypes with such a mutation are natural models for limb girdle muscular dystrophy 2B (22). Thus, the A/J strain is genetically prone to muscle weakness even when myasthenia is not induced with MuSK immunization. Intriguingly, the BALB/c strain is highly resistant to both anti-MuSK-antibody production and manifestations of muscle weakness after MuSK immunization (Table1, Figure 4); therefore, genetic factors other than the Dysf gene must play a role in the regulation of this autoimmune disease in mice. An association with HLA-DR14-DQ5 (odds ratio 8.5) was found in 23 Dutch Caucasians with MuSK-MG (23), thus one genetic factor determining susceptibility or resistance to this disease might be MHC-subclass II genes, which control antibody production in mice as well as humans. Additionally, MHC-subclass II (H-2A) genes mediate immune responsiveness to AChRs in mice with EAMG (24, 25), whereas AChR MG of humans is associated with polymorphism of the HLA-DQ genes (26)

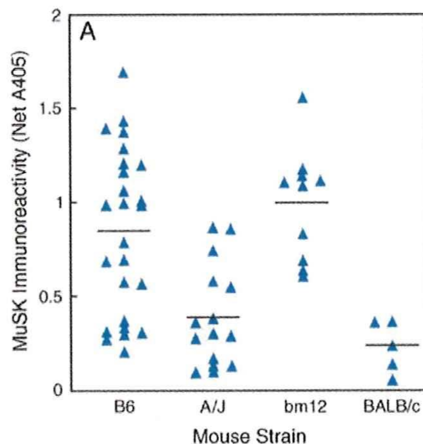

Figure 4. Immunoreactivity to MuSK of antisera (dilution,1:100,000) obtained after a second MuSK injection fromindividual mice. B6 and bm12 mice produced the largest amont of anti-MuSK antibodies, followed by A/J and then by BALB/c mice. Antisera obtained after a third injection showed similar results. (Reprinted from *J Neuroimmunol.* Jha et al., 175:107-117.2006 with permission from Elsevier.)

Although active immunization of rabbits and mice with the extracellular domain of MuSK protein clearly demonstrated the pathogenicity of anti-MuSK antibodies in EAMG (10-12), the failure to incite EAMG by passive transfer of human anti-MuSK antibodies into adult mice still challenged the idea that anti-MuSK antibodies can cause MG in humans. Previous studies showed that passive transfer of anti-AChR-negative sera from MG patients into adult mice did not reduce AChRs at the postsynaptic membrane

or cause myasthenia, but electrophysiological changes were present such as a reduction in miniature endplate potential amplitudes and endplate potential quantal content, which suggested the impairment of both pre- and post-synaptic transmission (5, 6, 27). In 2008, passive transfer of a large amount of IgG from MuSK MG patients into adult mice induced myasthenia with a significant reduction of AChR in the postsynaptic membrane and a decremental electromyographic trace on repetitive nerve stimulation (Figure 5) (13).

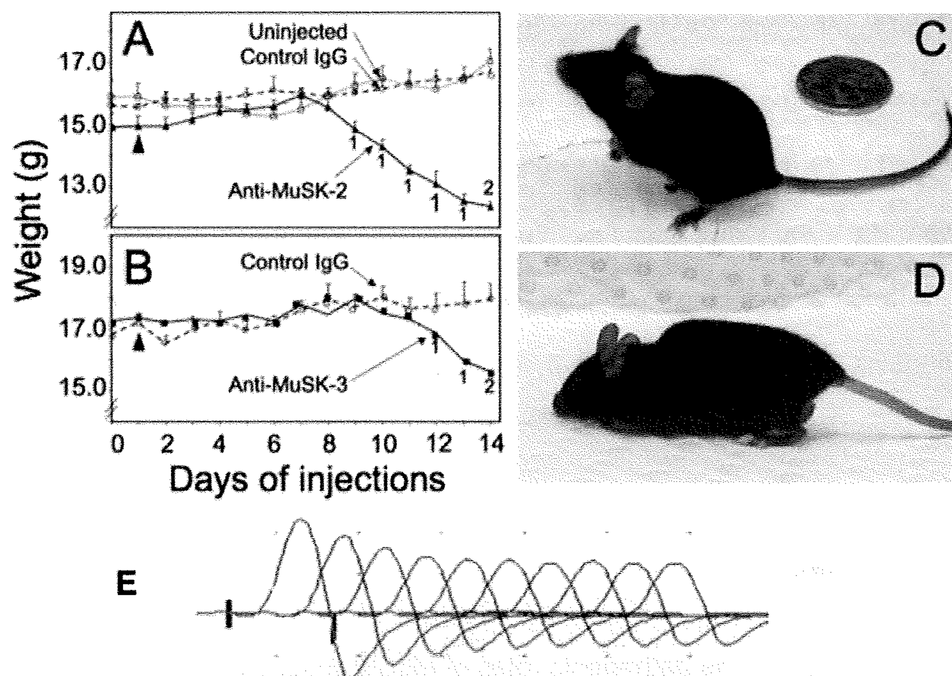

Figure 5. MG-like muscle weakness and weight loss in C57BL/6J mice after injection with anti- MuSK IgG purified from MuSK-MG patients. Mice injected with IgG from anti–MuSK–positive Patient 2 began to lose weight and develop muscle weakness from 9 to 12 days after the start of daily injections. Control IgG-injected and uninjected mice were not affected. Numbers beside symbols refer to the muscle weakness grading scale as described in Table 1. (B) In a separate experiment, mice injected with IgG from anti-MuSK–positive Patient 3 similarly lost weight and became weak. (C) Mouse after 14 days of injections with control IgG, showed no sign of weakness after the standard exercise regimen. Coin diameter was 28mm. (D) Mouse after 14 days of injections with IgG from anti-MuSK–positive Patient 2 showed muscle deterioration with chin down, flaccid tail and limb weakness. Note also the characteristic hump in the upper back. (E) Electromyography recorded from the gastrocnemius muscle of a C57BL/6J mouse injected for 14 days with IgG from anti–MuSK–positive Patient 2. Data represent means ± standard error of the mean for n=3 mice for all groups except for mice injected with Patient 3 IgG. (B); the amount of IgG available was limited, so n=2. (Reprinted with permission from Cole et al., *Ann Neurol* 63:782-789. 2008)

The inbred mice (C57BL/6J strains) were injected daily (intraperitoneally - IP) with 45mg human IgG from two patients (anti-MuSK-2; patient 2, and anti-MuSK-3; patient 3, in Figure 5A and B) for more than 5 days with a single IP injection of cyclophosphamide monohydrate 24 hours after the first IgG injection to suppress immune reactions against the human protein. After 14 days of injections, mice developed signs of weakness such as chin down, flaccid tail, and limb weakness with a prominent cervicothoracic hump (Figure 5D), which may reflect weak cervical extensor muscles. These features were also observed in mice with EAMG induced by MuSK immunization (11, 12). Mice injected with control IgG showed neither weight loss nor muscle weakness compared with uninjected mice (Figure 5C). Decremental electromyographies typical of MG patients were recorded in the mice injected with human anti-MuSK antibodies (Figure 5E). The evidence of EAMG from the passive transfers clearly demonstrated that anti-MuSK antibodies in MG patients cause their disease rather than being just bystander antibodies.

2. Structures of NMJ

Before we discuss the pathogenic roles of MuSK antibodies in EAMG and patients, we should look at the structure of NMJs and MuSK functions. NMJs are the sites of synapses between motor nerves and muscle fibers (Figure 6). The NMJ comprises portions of three cells such as motor neurons, muscle fibers and Schwann cells (28, 29). The motor nerve terminal is specialized for neurotransmitter (acetylcholine:ACh) release. Synaptic vesicles storing ACh adjacent to specialized structures of the presynaptic membrane are called active zones. The active zones are

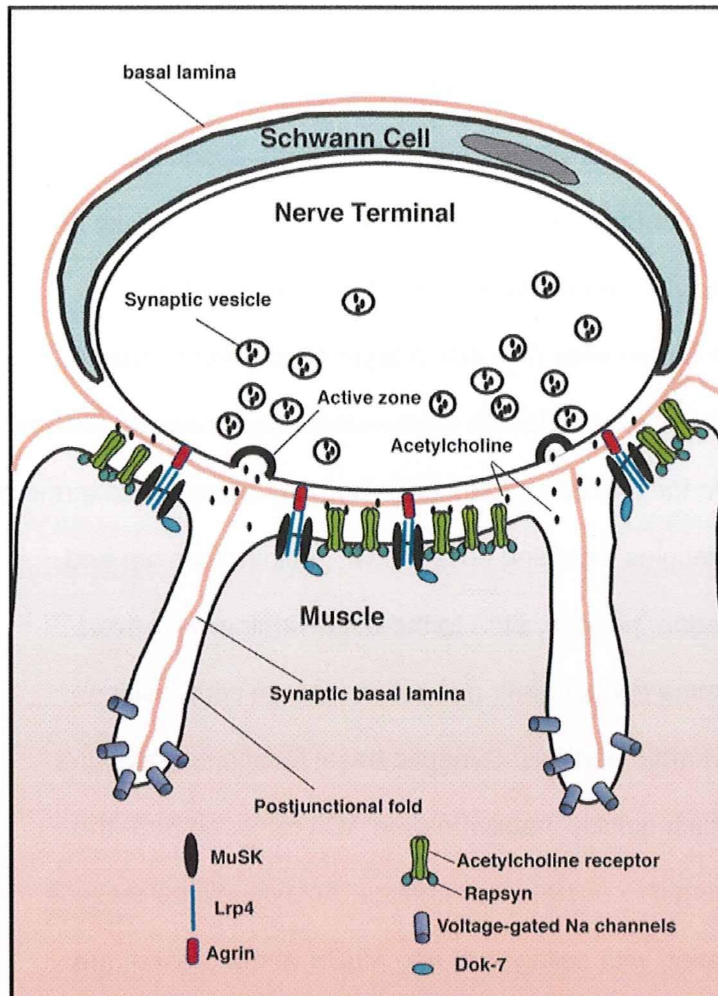

Figure 6. Structure and molecular architecture of NMJ. Details are discussed in the text.

precisely opposite mouths of the postjunctional folds (30, 31). AChRs are highly concentrated, with a density of about 12,000 receptors per μm^2(32), at the post-junctional membrane nearest the fold's peak (Figure 6) (33). Rapsyn is co-localized with AChRs as a scaffold molecule and required for the clustering of AChRs (34). When the nerve action potential reaches the terminal, depolarization opens voltage-gated Ca^+ channels on the presynaptic membrane (35, 36). This allows a Ca^+ influx that triggers the fusion of synaptic vesicles with the presynaptic membrane and the release of ACh

(37, 38). The postsynaptic membrane responds rapidly and dependably to ACh released from the overlying active zones in the nerve terminal. AChRs, by binding ACh, become transiently permeable to both Na^+ and K^+, then opening the associated voltage-gated ion channels in the depths of folds, which contribute to the action potential and muscle contraction (28). The synaptic cleft between nerve terminals and the postsynaptic membrane is about 50 nm wide (39, 40). A layer of connective tissue called basal lamina (basement membrane) sheaths each muscle fiber, passes through the synaptic clef and extends into the junctional folds (29). Both the presynaptic terminal and the muscle fiber secrete molecules including collagen IV, laminin, ectactin and heparan sulfate proteoglycans (agrin, perlcan, etc.) to the basal lamina. However, synaptic portions of the basal lamina contain their distinctive isoform composition separate from that of the extrasynaptic portions. Synaptic basal lamina contains the enzyme acetylcholinesterase, which quickly inactivates the ACh released from the presynaptic terminal by hydrolyzing it to acetate and choline. Acetylcholinesterase is clustered with AChRs by association with collagen Q and MuSK at the crest of the junctional folds (41). Concentrations of released ACh in the synaptic cleft decrease rapidly by diffusion and interaction with acetylcholinesterase, upon which the neuromuscular transmission terminates.

3. MuSK functions in NMJ

MuSK, which is a receptor-tyrosine kinase, is a component of the agrin receptor with the low-density-lipoprotein receptor-related protein 4 (LRP4) at the postsynaptic

membrane (Figure 6) (8, 42-46). MuSK knock-out mice display devastating defects in both pre- and post-synaptic differentiation and die at birth because they cannot breathe, thus MuSK requires the formation of NMJs during neuromuscular development (8). In the knock-out mice, motor axons grow excessively and fail to form terminal arbors, and no AChR-rich clusters are present on myotubes opposing ingrowing motoneuron terminals as shown in Figure 7. Since MuSK is expressed in skeletal muscle but not in motor neurons (42), MuSK requires regulation of the retrograde signals for differentiation of pre-synaptic structures in NMJs (8).

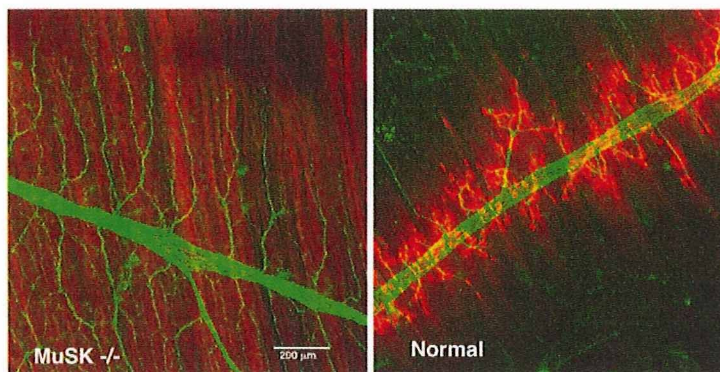

Figure 7. Aberrant structures of pre- and postsynaptic differentiation in diaphragm muscle from MuSK-/- mutant mouse. Motor axon (green), AChR(red).

MuSK also plays multiple roles in AChR clustering during development of the postsynaptic membranes of NMJs (47, 48). Contact of the motor-nerve growth cone with the muscle induces a narrow, distinct endplate zone in the mid-muscle that is marked by a high density of AChR clustering. In this step, innervation disperses aneural AChR clusters in nonsynaptic areas via muscle activity elicited by ACh (49, 50), whereas in synaptic regions, this negative activity is overpowered by agrin delivered by motor nerve terminals to induce AChR clusters (44, 48). However, since agrin does not bind MuSK, additional components are required to activate MuSK (44, 47). Recent

studies demonstrated that LRP4, a member of the LDLR family, is a long-sought receptor of agrin, forms a complex with MuSK, and mediates MuSK activation by agrin (45, 46). MuSK is also required for organizing a primary synaptic scaffold to create the post-synaptic membrane (48). Prior to muscle innervation, AChR clusters form at the central regions of muscle fibers, creating an endplate zone that is somewhat broader than that in innervated muscle (51, 52). Thus, MuSK is required for pre-patterning of AChR clustering in the absence of motor innervation. An intriguing finding is that MuSK has a cysteine-rich domain that shows homology to the Wnt receptor Frizzled (53, 54). Wnt is a family of secreted proteins that are implicated in neural development, neural outgrowth, navigation, and synaptogenesis (55-60). Wnt ligans bind to the receptor complex of Frizzled and LRP5/6 (61). Subsequently, signal is transmitted to the adaptor protein Dishevelled (Dvl), which interacts with Frizzled, to initiate intracellular canonical and noncanonical pathways. Like Frizzled, MuSK might interact on the membrane with LRP4 as a Wnt receptor and promote postsynaptic specialization at mammalian NMJ as in Zebrafish (62, 63). In fact, pre-patterning of AChR clustering disappears in LRP4 mutant mice as MuSK-deficient mice (46). Intriguingly, a number of Wnt signaling molecules including APC, β-catenin, Dvl have been implicated in MuSK signaling (63). Simultaneously or alternatively, MuSK could, thereby, form a primary scaffold molecule without activation by agrin.

The listed pleiotropic roles of MuSK in AChR clustering at developmental NMJs could also be required for the maintenance of mature NMJs throughout life. Studies performed *in vivo* have shown that synaptic AChRs intermingle among themselves completely over a period of ~four days and that many extra-synaptic AChRs are