Large^{myd}マウス

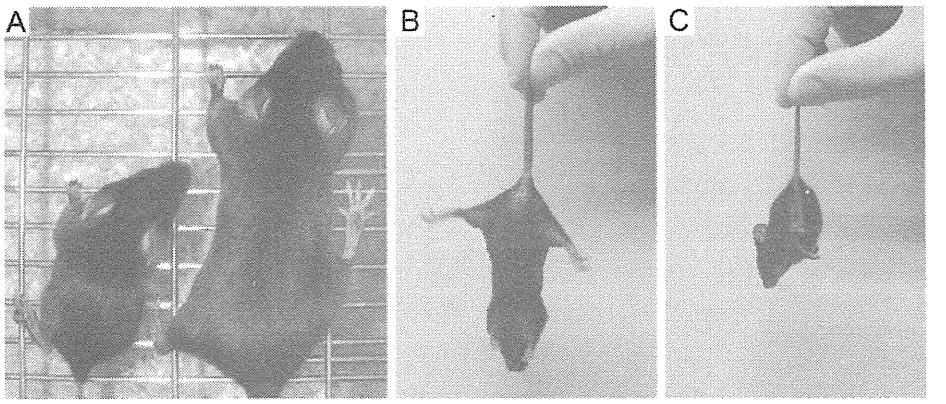
【系統名】B6C3Fe a/a-Large^{myd}/J
【背景系統】B6C3Fe-a/a
【系統の分類】mutant stock
【繁殖・維持】homozygous for Large^{myd}×B6C3Fe-a/a hybrid（female×male）．繁殖は，変異ホモとヘテロでも可能だが，ヘテロ同士の掛け合わせに比べて，妊娠率は低下
【作製の背景】自然発症．*large*遺伝子のエクソン5～7の欠失によるフレームシフト

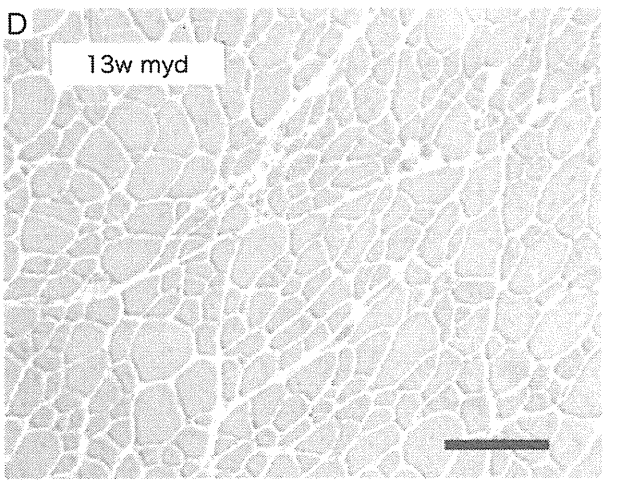
❑ 特徴

*myd*マウスは，重症の先天性筋ジストロフィータイプ1D（CMD1D）のモデルマウスであり，進行性の筋ジストロフィー，眼の異常，大脳皮質，小脳，海馬での神経細胞の遊走異常と基底膜の亀裂を特徴とする中枢神経系の異常を起こす．muscle-eye-brain病（MEB病）や福山型先天性筋ジストロフィー（FCMD）患者と同様に，骨格筋と脳でα-dystroglycan（α－DG）の糖鎖修飾低下が観察される．野生型・ヘテロの同腹マウスよりも小さく，歩行異常やクラスピングも認められる（図）．largeを過剰発現させると，POMGnT1やFukutinが欠損する筋細胞でもα-DGの糖鎖修飾が回復し，マウスでは筋ジストロフィーの表現型が改善することが知られており，治療への応用が期待される．

❑ 用途

α-DGの糖鎖修飾異常による先天性筋ジストロフィーの病態研究・治療研究．





❖ mydマウス
A～C）mydと同腹の野生型マウス（5週齢）．C）mydは身体が小さく，尻尾を持ち上げると，四肢を抱え込むようなクラスピングがみられる．D）13週齢mydマウスの骨格筋のHE染色．筋壊死と再生像，細胞浸潤，筋線維の大小不同，中心核線維等がみられる

❑ 補足

【注意点】*large*のコードするタンパク質はおそらくα-DGの糖鎖修飾に関与すると思われるが，その機能は明らかではない．他に，α-DGの糖鎖修飾の異常をきたす疾患のモデルマウスとして，POMGnT1-null mice（MEB病のモデル）や，FCMD患者の変異を導入したマウスが，作出されており，α-DGの糖鎖修飾の異常による筋ジストロフィーの病態解明，治療法の開発に用いられる

❑ 入手方法

【入手先】Jackson研究所（http://jaxmice.jax.org/strain/000226.html；MGI：1856965），あるいは日本チャールス・リバー社（http://www.crj.co.jp/）から入手可能．特にライセンス等の制限なし

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➡Large^{myd}の表現型と糖鎖異常の解析

β-sarcoglycanノックアウトマウス〔BSG-KO（C57BL/6J）〕

【系統名】B6.129S4-Sgcb<tm1Oza>，理研BRC系統番号 #RBRC02268
【系統の分類】戻し交配および戻し交配後の兄妹交配
【背景系統】C57BL/6JJcl
【作製の背景】129/SvJ系統のJ1 ES細胞を用いて，標的遺伝子組換え法により欠損マウスを作製し，C57BL/6JJcl系統に10世代以上戻し交配を行った

❑ 特徴・注意点

dystrophin複合体の構成分子のβ-sarcoglycanの欠損マウス．ホモ変異マウスは，筋力低下，筋肥大，骨格筋と心筋に著しい筋壊死と再生を伴う筋ジストロフィーの所見を示す．

❑ 用途

肢帯型筋ジストロフィー（LGMD2E）の病態解明，治療方法開発の基礎研究．脳神経系における機能の解明研究．

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3）理研バイオリソースセンターの本モデルのページ（http://www2.brc.riken.jp/lab/animal/detail.php？brc_no＝RBRC02268&skey＝%BA%FB%B2%AC&keito＝&dna＝&brcno＝）

γ-sarcoglycanノックアウトマウス〔GSG-KO（C57BL/6J）〕

【系統名】B6.129S4-Sgcg<tm1Oza>，理研BRC系統番号 #RBRC02269
【系統の分類】戻し交配および戻し交配後の兄妹交配
【背景系統】C57BL/6JJcl
【作製の背景】129/SvJ系統のJ1 ES細胞を用いて，標的遺伝子組換え法により欠損マウスを作製し，C57BL/6JJcl系統に10世代以上戻し交配を行った

❑ 特徴・注意点

dystrophin複合体の構成分子のγ-sarcoglycanの欠損マウス．ホモ変異マウスは，筋力低下，筋肥大，骨格筋と心筋に著しい筋壊死と再生を伴う筋ジストロフィーの所見を示す．

❑ 用途

肢帯型筋ジストロフィー（LGMD2C）の病態解明，治療方法開発の基礎研究.

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caveolin-3ノックアウトマウス

【系統名】 cav3-KO/B6（177）（B6/Jに戻し交配10世代）．B6.129S4-Cav3<tm1Ncnp>，理研BRC系統番号 #RBRC02374

【系統の分類】 戻し交配および戻し交配後の兄妹交配

【背景系統】 C57BL/6JJcl

【作製の背景】 129/SvJ系統のJ1 ES細胞を用いて，標的遺伝子組換え法により欠損マウスを作製し，C57BL/6JJcl 系統に10世代以上戻し交配を行った

❑ 特徴・注意点

dystrophin複合体の構成分子のcaveolin-3の欠損マウス．ホモ変異マウスは，骨格筋に筋壊死と再生を伴う筋ジストロフィーの所見を示す．骨格筋のカベオラ構造の減少を示す.

❑ 用途

筋ジストロフィーの病態解明，治療方法開発の基礎研究．心臓における標的遺伝子の機能解明研究．

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ADVANCES IN MOLECULAR THERAPY RESEARCH ON DYSTROPHIN-DEFICIENT MUSCULAR DYSTROPHY*

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Duchenne muscular dystrophy (DMD) is a lethal muscle disorder caused by mutations of the *DMD* gene, which encodes a 427-kDa spectrin-like cytoskeletal protein, dystrophin. Exon skipping induced by antisense oligonucleotides is a novel method to restore the reading frame of the mutated DMD gene and rescue dystrophin expression. We recently demonstrated that systemic delivery of antisense phosphorodiamidate morpholino oligonucleotides (PMOs) targeting exons 6 and 8 of the canine *DMD* gene efficiently recovered functional dystrophin at the sarcolemma of dystrophic dogs, and improved performance of the affected dogs without serious side effects. As a strategy to target hot spots of mutation in the *DMD* gene, we also tried exon 51-skipping using PMOs in *mdx*52 mice to convert an out-of-frame mutation into an in-frame mutation with restoration of dystrophin expression in various muscles and improvement of pathology and function. Progress in adeno-associated virus vector serotype 9 (AAV-9)-mediated DMD gene therapy has enabled the delivery of the therapeutic gene to the whole musculature, including cardiac muscle, while evoking minimal immunological reactions in mice, dogs, and non-human primates. Furthermore, DMD-derived patient-specific induced pluripotent stem (iPS) cells could be a potential source for cell therapy, although there are at present hurdles to be overcome. In the future, this technology could be used in combination with exon skipping or AAV-mediated gene therapy to achieve clinical benefits.

Keywords: Duchenne muscular dystrophy; exon skipping; AAV vector; iPS cells.

Duchenne Muscular Dystrophy

Duchenne muscular dystrophy (DMD) is the most common form of childhood muscular dystrophy. DMD is an X-linked recessive disorder with an incidence of 1 in

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3500 live male births (Emery, 1991). DMD causes progressive degeneration and regeneration of skeletal and cardiac muscles due to mutations in the dystrophin gene, which encodes a 427-kDa subsarcolemmal cytoskeletal protein (Hoffman *et al.*, 1987). DMD is associated with severe, progressive muscle weakness and typically leads to death between the ages of 20 and 35 years. Due to recent advances in respiratory care, much attention is now focused on treating the cardiac conditions suffered by DMD patients. Although various new therapeutic strategies, including new drug design, gene therapy and cell therapy, have been proposed, no effective treatment has yet been established (Table 1).

Impact of Exon-Skipping Therapy

Exon-skipping therapy is detailed in our previous reviews (Nakamura and Takeda, 2009; Miyagoe-Suzuki and Takeda, 2010). Here, we present an overview of the most specific impact of our approach. Dystrophin is essentially missing at the sarcolemma in DMD, although some dystrophin-positive fibers, called revertant fibers, are present. The number of revertant fibers increases with age due to the cycle of degeneration and regeneration (Wilton *et al.*, 1997). It is currently thought that the molecular mechanism underlying the revertant fibers is the skipping of the exon

Table 1. Clinical trials for DMD/BMD.*

Category	Drug/Genetic	Phase (ClinicalTrials.gov)
Drug interventions	Myostatin blocker	
	MYO-029	Completed; not effective
	Read-through	
	PTC124	Completed; not effective (Phase IIb)
	Gentamicin	Completed; not effective
	Others	
	Pentoxifylline	Completed; not effective
	Idebenone	Phase III
	Ramipril vs. Carvedilol	Phase VI
	CoQ10 and prednisone	Phase III
	Coenzyme Q10 and lisinopril	Phase II/III
	Debio-025 (cyclosporine analogue)	Phase IIb
Gene therapy	Exon skipping (systemic delivery)	
	PRO051 (21'-*O*-MePS AO)(exon 51 skipping)	Phase III
	PRO044 (2'-*O*-MePS AO)(exon 44 skipping)	Phase I/II
	AVI-4658 (PMO)(exon 51 skipping)	Phase IIb
	AAV vector	
	rAAV2.5-CMV-Mini-Dystrophin	Phase I
Cell therapy	Satellite cells (myoblasts)	Pending
	Mesoangioblasts	In preparation
	Mesenchymal stem cells	Ongoing
	Induced pluripotent stem (iPS) cells	Experimental

*Detailed information can be retrieved by using a registry of the clinical trials conducted in the United States and around the world (http://www.clinicaltrials.gov).

around the original mutation, which gives rise to the correction of the reading frame and expression of dystrophin. Consequently, exon skipping has attracted attention as a strategy for restoration of dystrophin expression in DMD. To induce skipping of specific exons during mRNA splicing, antisense oligonucleotides (AOs) against exonic and intronic splicing regulatory sequences have been generated. This use of these AOs has been shown to correct the open reading frame of the DMD gene and thus to restore truncated yet functional dystrophin expression *in vitro* (Takeshima *et al.*, 1995). In order to overcome the unstable disposition of single-stranded DNA or RNA, the AOs were designed using various chemical modifications of natural nucleic acid structure (Fig. 1).

Intravenous infusion of an antisense phosphorothioate oligonucleotide created an in-frame dystrophin mRNA via exon skipping in a 10-year-old DMD patient possessing an out-of-frame exon 20 deletion of the *dystrophin* gene (Takeshima *et al.*, 2006). Also, the adverse-event profile and local dystrophin-restoring effects of a single intramuscular injection of an antisense 2'-O-methyl phosphorothioate oligonucleotide (2OMeAO), PRO051, in patients with DMD were explored (Table 1). Four patients received a dose of 0.8 mg of PRO051 in the tibialis anterior (TA) muscle. Each

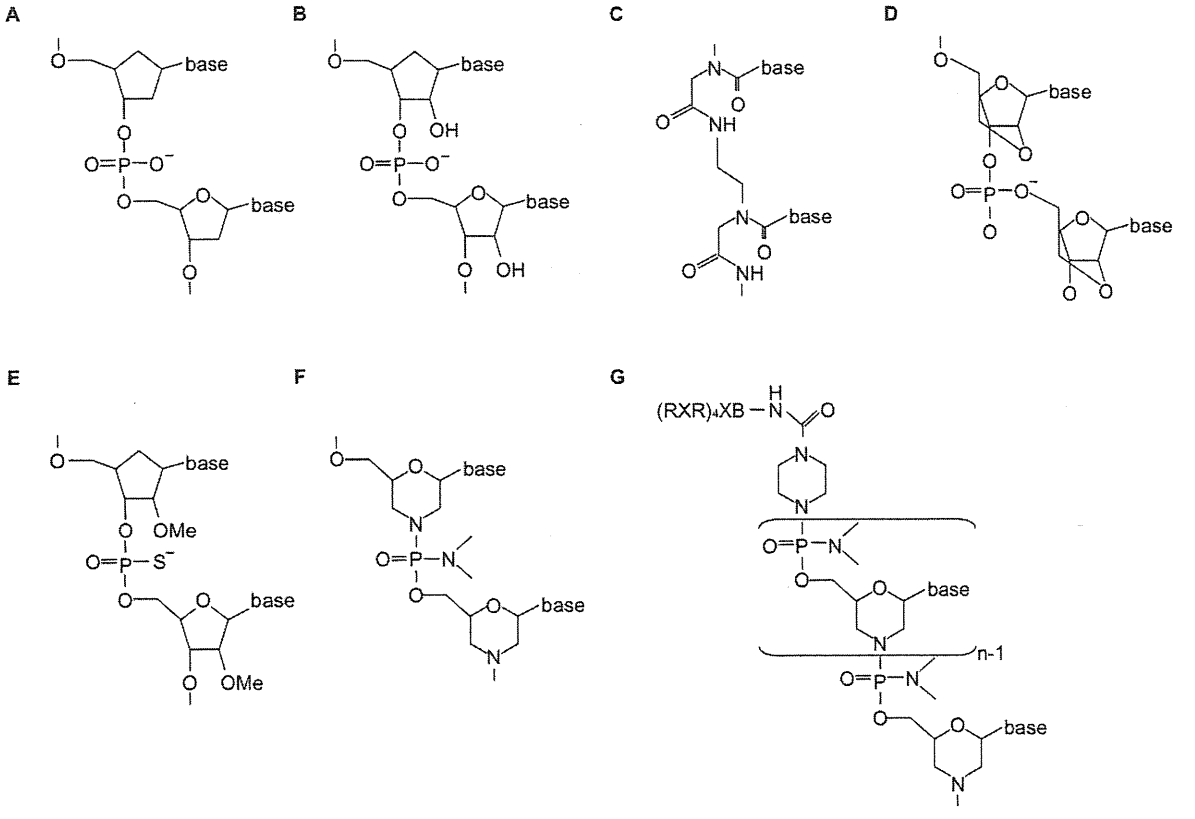

Fig. 1. Nucleic acid variations used for exon skipping. (redrawn from Nakamura and Takeda, 2009, with modifications). A, DNA; B, RNA; C, peptide nucleic acid; D, ethylene-bridged nucleic acid; E, 2′-O-methyl phosphorothioate oligonucleotide; F, phosphorodiamidate morpholino oligomer (PMO); G, peptide-linked PMO (PPMO).

patient showed specific skipping of exon 51 of dystrophin in 64 to 97% of myofibers, without clinically apparent adverse side effects (van Deutekom *et al.*, 2007).

Dystrophin-deficient canine X-linked muscular dystrophy was found in a golden retriever with a 3′ splice-site point mutation in intron 6 (Valentine *et al.*, 1988). The clinical and pathological characteristics of dystrophic dogs are more similar to those of DMD patients than are those of *mdx* mice. A beagle-based model of canine X-linked muscular dystrophy, which is smaller and easier to handle than the golden retriever-based muscular dystrophy dog (GRMD) model, has been established in Japan, and is referred to as $CXMD_J$ (Shimatsu *et al.*, 2005). The limb and temporal muscles of $CXMD_J$ show the effects of the disease starting at two months of age, which is the age corresponding to the second peak of serum creatine kinase. Using distinct multi-antisense oligonucleotides, we induced multi-exon skipping in $CXMD_J$ through muscular and intravenous injection of stable phosphorodiamidate morpholino oligonucleotides (PMOs). Treatment of the $CXMD_J$ dogs with the three PMOs targeting exons 6 and 8 resulted in the widespread rescue of dystrophin expression to therapeutic levels without signs of prominent toxicity (Yokota *et al.*, 2009).

Among the mutation hot spots, patients with various deletions within exons 45–55 account for 60% of DMD patients. We optimized the sequences of antisense PMOs targeting exon 51 of the mouse *DMD* gene to prepare for clinical trials involving patients with these hot-spot mutations. We designed 14 kinds of antisense PMOs targeting exon 51 of the mouse *DMD* gene and injected them separately or in combination into the muscles of *mdx*52 mice, in which exon 52 had been deleted by a gene targeting technique (Araki *et al.*, 1997). A combination of two PMOs showed an excellent restoration of sarcolemmal dystrophin in the injected muscle (Fig. 2;

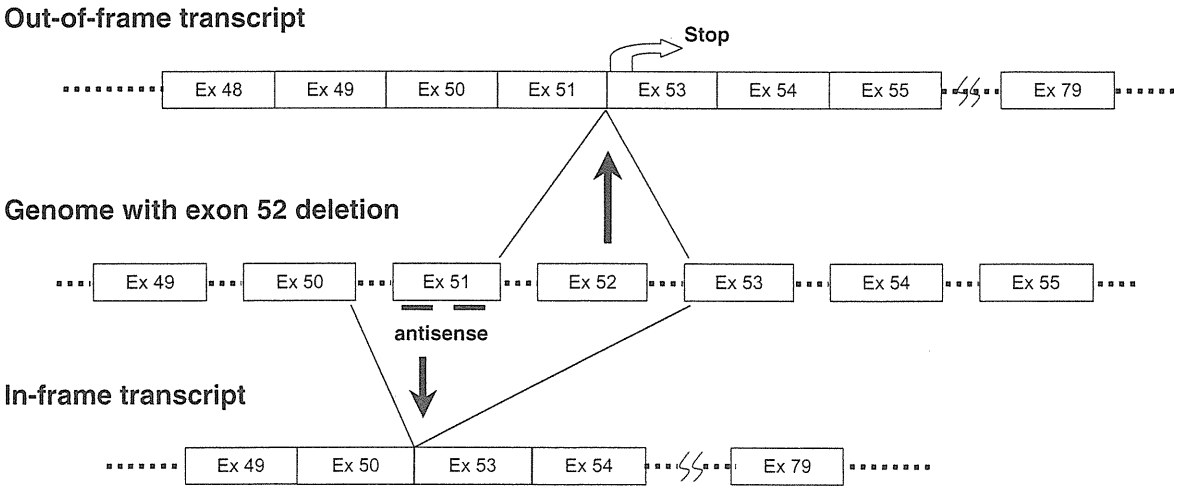

Fig. 2. Exon 51 skipping in the *mdx*52 mouse. Exon 52 (Ex 52) has been deleted in *mdx*52 mice and thus *mdx*52 mice harbor out-of-frame transcripts with a premature stop codon. The antisense oligomers targeting exon 51 (Ex 51) would induce exon 51 skipping, resulting in the production of in-frame transcripts.

Aoki *et al.*, 2010). We then continued to intravenously inject these PMOs into the *mdx*52 mice seven times weekly. Two weeks after the final injection, dystrophin was expressed at the sarcolemma throughout the body at an average of 10–50% of normal levels. This was accompanied by amelioration of dystrophic pathology as well as improvement in the contractile force of extensor digitorum longus muscle, grip power test, and treadmill performance. This study provides proof of concept for exon 51 skipping in a DMD animal model that can be applicable to up to 15% of *DMD*-deletion patients. Various unrelated patients with deletions of exons 45–55 harboring mild or asymptomatic skeletal muscle involvement have been reported (Beroud *et al.*, 2007; Nakamura and Takeda, 2009). If multi-exon skipping of exons 45–55 is possible, severe Becker muscular dystrophy (BMD) cases with a deletion in the hot spot may also be treatable. We have been currently investigating the use of mixtures of PMOs to confirm the feasibility of therapy based on exon 45–55 skipping.

One of the issues remaining for exon-skipping therapy is inconsistency of efficacy among different organs or tissues. For instance, systemic administration of AOs restored dystrophin expression, but expression levels were much lower in cardiac muscle compared with skeletal muscle (Yokota *et al.*, 2009). Since a number of DMD patients die of cardiac complications, including lethal arrhythmias, improvement of AO transfer in the heart is critical. It is still not clear why AO uptake into cardiac tissue is inefficient. Since cardiac muscle cells each harbor a single nucleus, in contrast to the multinucleated skeletal muscle cells, damaged cardiomyocytes may be immediately replaced with fibrous tissues before uptake of AOs (Nakamura and Takeda, 2009). To improve introduction efficiency into the heart, PMO injection using a microbubble contrast reagent under ultrasound has been proposed (Wang *et al.*, 2005). Also, PMOs with a designed cell-penetrating peptide have been developed to efficiently target a mutated *dystrophin* exon in cardiac muscles (Wu *et al.*, 2008). Long-term benefits can be achieved through the use of viral vectors expressing antisense sequences against regions within the *dystrophin* gene. The sustained production of dystrophin at physiological levels in entire groups of muscles as well as the correction of muscular dystrophy could be achieved by treatment with exon-skipping adeno-associated virus (AAV)-U7 (Goyenvalle *et al.*, 2004).

Gene-replacement Strategies using Adeno-Associated Virus Vectors

AAV-mediated gene therapy for DMD was updated in our previous review (Miyagoe-Suzuki and Takeda, 2010). Here, we highlight our own AAV-based effort aimed at expressing transgenic dystrophin in DMD patients. While various viral vectors have been considered for the delivery of genes to muscle fibers, the AAV-based vector is emerging as the gene transfer vehicle with the most potential for use in DMD gene therapy. The advantages of the AAV vector include the lack of pathology associated with the wild-type virus, the ability to transduce non-dividing cells,

and the long-term expression of the delivered transgene (see: Okada *et al.*, 2002). Serotypes 1, 6, 8, and 9 of recombinant AAV (rAAV) exhibit a potent tropism for striated muscles (Inagaki *et al.*, 2006). Since a 5-kb genome is considered to be the upper limit for a single AAV virion, a series of rod-truncated micro-dystrophin genes is used in this treatment (Yuasa *et al.*, 1998). To gain acceptance as a medical treatment, AAV vectors require a scalable and economical production method. A scalable method, using active gassing and large culture vessels, was developed to enable large-scale plasmid transfection in a closed system (Okada *et al.*, 2005). Recent developments in ion-exchange chromatography also suggest that vector production using transduction culture supernatant would be compatible with current good manufacturing practice and production on an industrial scale (Okada *et al.*, 2009).

Although recent studies suggest that vectors based on AAV are capable of body-wide transduction in rodents, translating this finding into large animals remains a challenge. Since increased permeability of the sarcolemma allows leakage of transgene products from the dystrophin-deficient muscle fibers, neo-antigens introduced by AAV vectors evoke significant immune reactions in DMD muscle (Yuasa *et al.*, 2002). Furthermore, an *in vitro* interferon-gamma release assay showed that canine splenocytes respond to immunogens or mitogens more strongly than do murine splenocytes. In fact, co-administration of the immunosuppressants cyclosporine (CSP) and mycophenolate mofetil (MMF) improved rAAV2 transduction. Immunohistochemical analysis revealed that the rAAV2-injected muscles showed higher rates of infiltration of CD4+ and CD8+ T lymphocytes in the endomysium than the rAAV8-injected muscles (Ohshima *et al.*, 2009). Our study also showed that mRNA levels of MyD88 and co-stimulating factors, such as CD80, CD86 and type I interferon, are elevated in both rAAV2- and rAAV8-transduced dog dendritic cells (DCs) *in vitro* (Ohshima *et al.*, 2009). Intravascular delivery can be performed as a form of limb perfusion, which might bypass the immune activation of DCs in the injected muscle. Administration of rAAV8-micro–dystrophin by limb perfusion produced extensive transgene expression in the distal limb muscles of $CXMD_J$ dogs without obvious immune responses for as long as eight weeks after injection (Ohshima *et al.*, 2009).

It is increasingly important to develop strategies to treat DMD that consider their effects on cardiac muscle. The pathology of the conduction system in $CXMD_J$ dogs was analyzed in order to establish a suitable therapeutic target for DMD (Urasawa *et al.*, 2008). Although dystrophic changes of the ventricular myocardium were not evident at the age of 1 to 13 months, Purkinje fibers showed remarkable vacuolar degeneration when dogs were as young as four months old. Because the dystrophin-deficient heart is highly sensitive to increased stress, increased activity by the repaired skeletal muscle provides the stimulus for heightened cardiac injury and heart remodeling. A single intravenous injection of AAV9 vector expressing micro-dystrophin efficiently transduces the entire heart in neonatal *mdx* mice, thereby ameliorating cardiomyopathy (Bostick *et al.*, 2008). The systemic delivery

of rAAV to transduce truncated dystrophin is predicted to ameliorate the symptoms of DMD patients. To realize gene transduction technologies in clinical practice, development of an effective delivery system with improved vector constructs as well as efficient immunological modulation must be established.

Prospects for Stem Cell Therapy and iPS Cells-mediated Gene Therapy

Stem cells-based cell therapy for DMD is another approach on which we are also focusing. Although initiated by intramuscular injection of allogeneic myoblasts in DMD patients (Law *et al.*, 1992; Huard *et al.*, 1992; Gussoni *et al.*, 1992; Karpati *et al.*, 1993), our ultimate goal is to deliver therapeutic stem cells into skeletal muscles via the vascular system as pioneered by Gussoni *et al.* with haematopoietic and muscle-derived stem cells on the *mdx* mouse model (1999). The main challenge is the identification and availability of the stem cell type able to efficiently perform this task. Mesoangioblasts are attractive candidates (Sampaolesi *et al.*, 1996) together with muscle satellite stem cells (Zammit *et al.*, 2002) and mesenchymal stem cells (MSCs) on which we have already a direct interest (Uezumi *et al.*, 2010; Kasahara *et al.*, 2010). However, we believe that patient-specific induced pluripotent stem (iPS) cells offer a unique therapeutic opportunity for DMD.

Since the breakthrough reprogramming of mouse (Takahashi and Yamanaka, 2006) and human (Takahashi *et al.*, 2007; Yu *et al.*, 2007) somatic cells into iPS cells, patient-specific iPS cells have been produced as disease-specific cellular models including DMD and BMD (Park *et al.*, 2008) and are envisioned as an obvious drive both for regenerative medicine (see Yamanaka, 2009) and stem cell gene therapy (e.g. Hanna *et al.*, 2007; Raya *et al.*, 2009). Of note, a first step in the proof-of-concept for iPS cells-mediated gene therapy for DMD has been recently published in which a human artificial chromosome has been used to correct iPS cells derived from a DMD patient and from *mdx* mice (Kazuki *et al.*, 2010). On the other hand, using directed differentiation and anti-satellite cell antibodies, stem/progenitor cells have been derived from wild-type mouse iPS cells that successfully engrafted in *mdx* mice (Mizuno *et al.*, 2010). The iPS cell breakthrough is thus moving straightforwardly toward the clinic. However, major safety concerns have still to be cleared before the initiation of the bench-to-bedside translation (Yamanaka, 2009).

Conclusions

The combination of gene and cell therapy is an optimal way to allow patient-specific custom-made therapies. Both gene and cell therapies for DMD have demonstrated promising results in animals, although their promise has not yet been translated into success in human patients. Investigations in large animal models are clarifying why it is hard to move from rodents to humans and are at the same time implying various possible solutions to these difficulties. The key to future accomplishment

would be cautious preclinical studies combined with well-planned clinical trials that concentrate on safety and efficacy issues.

Acknowledgments

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Chapter 4
Mechanobiology in Skeletal Muscle: Conversion of Mechanical Information into Molecular Signal

Yuko Miyagoe-Suzuki and Shin'ichi Takeda

4.1 Introduction

Overload leads to muscle hypertrophy. In the process, several events occur inside and outside the myofibers, including increased protein synthesis, change in gene expression, fiber-type transition, satellite cell activation, and angiogenesis (Bassel-Duby and Olson 2006; Blaauw et al. 2009). Interestingly, at the early phase of muscle hypertrophy, protein synthesis significantly increases (Baar et al. 2006), and later the transcription of growth-related genes follows (Carson 1997). Satellite cell activation is generally thought to be a critical component for increase in muscle mass; however, it is still a debated issue whether satellite cell incorporation into hypertrophying muscle fibers is required for muscle hypertrophy (O'Connor and Pavlath 2007; McCarthy and Esser 2007). A recent paper demonstrated that the rapid incorporation of BrdU seen in overloaded muscle reflects angiogenesis but not proliferation of satellite cells (Blaauw et al. 2009). Thus, muscle hypertrophy is a complicated and dynamic process, influenced by many factors (nutrients, blood flow, hormones, energy status, or oxidative status), making it difficult to elucidate the mechanism by which mechanical information is sensed by myofibers. IGF-1, anabolic steroids, or blockage of myostatin signaling increases muscle mass without mechanical stimulation via distinct mechanisms from mechanotransduction. For myostatin or steroids, please refer to other comprehensive reviews (Joulia-Ekaza and Cabello 2007; Kadi 2008).

The muscle atrophy process is characterized by suppressed protein synthesis, and increased rate of degradation of muscle proteins (Jackman and Kandarian 2004; Ventadour and Attaix 2006). Decreased muscle activity (i.e., denervation, prolonged bed rest, space flight, immobilization, etc.) and diseases (cancer, AIDS, muscular dystrophy, sepsis, chronic heart failure, diabetes, etc.), malnutrition, or drugs (glucocorticoids) lead to muscle atrophy. In chronic diseases, elevated levels

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of proinflammatory cytokines, glucocorticosteroids, tumor-derived factors, and endotoxins are responsible for the induction of muscle atrophy, whereas the upstream mediators which trigger catabolic signal cascades in unloaded muscle are largely unknown.

4.2 Mechanosensor in Skeletal Muscle

4.2.1 Experimental Models for Mechanotransduction

Experiments to study mechanotransduction in skeletal muscle employ in vivo and in vitro models (Table 4.1). As discussed below, experimental designs greatly influence the responses of the muscles to the mechanical stress.

Although it is not yet a consensus, it is reported that eccentric contraction more effectively activates p70S6K than centric contraction (Eliasson et al. 2006; Nader and Esser 2001). This difference might be because eccentric contraction accompanies stretching stimuli, and thereby strongly stimulates stretch-activated channels (SACs) or other stretch-sensitive mechanoreceptors. But this hypothesis needs more investigation.

In addition to the type of mechanical stimulation, the intensity and duration of stimulation must be taken into account on interpretation. Atherton et al. reported that aerobic exercise stimuli promoted specific adaptive responses toward mitochondrial biogenesis besides slow phenotypes, without activating mTOR and p70S6K. Resistance strength training stimuli, however, strongly activated mTOR and p70S6K (Atherton et al. 2005). But anther group did not observe such a difference (Nader and Esser 2001). As Zanchi and Lancha discuss in their review article (Zanchi and Lancha 2008), the difference might be due to the difference in the design of the experiment, i.e., in vivo versus in vitro, or in the duration of mechanical stimulation.

In overloaded muscle, the protein synthesis is initiated by mechanical stimuli, but later modulated by additional anabolic stimuli. IGF-1 is generally accepted to strongly

Table 4.1 Models to study mechanotransduction in skeletal muscle

Modes of mechanical load	
Overload	In vivo model
	Synergist ablation (functional overload)
	Electrical stimulation
	In vitro model
	Electrical stimulation in vitro
	Cell culture (stretch)
Unload	In vivo models
	Limb immobilization
	Hindlimb suspension
	Denervation

promote protein synthesis through the PI3K/Akt/mTOR pathway (Rommel et al. 2001; Bodine et al. 2001). Nonetheless, IGF-1-mediated PI3K/Akt/mTOR activation seems to be part of a late component of the hypertrophy process (Hameed et al. 2003; Haddad and Adams 2002). Furthermore, acute stimulation of skeletal muscle seems not to be always dependent on PI3K/Akt as a means to activate mTOR (Hornberger et al. 2004, 2006). Thus, acute phase might be more suitable than chronic phase to investigate the mechanotransduction system in skeletal muscle.

Lastly, skeletal muscle is composed of myofibers with different contractile and metabolic properties. For example, the soleus, which is tonic and postural, is a mixture of predominant oxidative slow-twitch (type I) fibers and glycolytic fast-twitch fibers (type II). The extensor digitorum longus (EDL) muscle, which is phasic, is composed of fast-twitch glycolytic fibers. Importantly, these two muscles respond to mechanical stimuli differently: the soleus shows a lower susceptibility to mechanical load compared with EDL (Widrick et al. 2002). The findings suggest either that different mechanosensing apparatus exists in these two muscles, or that different signal transduction pathways operate downstream of the mechanosensor in fast and slow muscles.

4.2.2 Mechanosensors in Skeletal Muscle

So far, many molecules have been proposed as a mechanosensing molecule in skeletal muscle, including SACs (Spangenburg and McBride 2006), the dystrophin-glycoprotein complex (Acharyya et al. 2005; Suzuki et al. 2007), integrins (discussed in Zanchi and Lancha 2008; Spangenburg 2009) or sarcomere structure (Gautel 2008) (Fig. 4.1), but none of them is definitive. It is quite plausible that skeletal muscle has multiple mechanosensors and integrates the mechanical information from all these sensors into anabolic or catabolic responses.

4.2.3 Stretch-Activated Channels

SACs were initially described in skeletal muscle (Franco and Lansman 1990a, b). SACs in skeletal muscle are permeable to both Na+ and Ca2+ and increase their open probability in response to stretch of the membrane. A previous report, using Ga3+ and streptomycin as specific blockers of SACs, suggested that SACs are activated during lengthening or stretch-induced contraction, activate the Akt/mTOR pathway, and induce muscle hypertrophy (Spangenburg and McBride 2006; Butterfield and Best 2009). However, since neither Ga3+ nor streptomycin completely abolished Akt/mTOR activities, the authors speculated that SACs is one of the molecules which sense mechanical load and promote protein synthesis. Currently, SACs are identified based on patch-clamping experiments, and channel activity is blocked with specific inhibitors. Cloning of genes encoding SACs and