Muscular Dystrophy: From Pathogenesis to Strategy

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Abstract- Muscular dystrophies are a genetically heterogeneous group of degenerative muscle disorders. It characterized by progressive muscle wasting and weakness of variable distribution and severity. There are several subgroups including Duchenne/Becker, fascioscapulohumeral, limb-girdle, oculopharngeal, and congenital muscular dystrophy. Diagnosis is dependent to the characteristic clinical features in distribution of predominant muscle weakness, disease course and age onset as well as variable serum concentration creatine kinase, muscle histology, and genetic inheritance. Nearly 30 genes and encoded proteins are known to give rise to various forms of muscular dystrophy. Development of new prospects therapy for the muscular dystrophies is a big challenge. The target of strategies is aimed at inducing of a functional protein and improving the function of muscle weakness. These strategies include gene, cell and pharmacological therapies. However, efficiency of systemic delivery vectors to targets, immune reaction to vector and gene products, and toxicity to vector that must be solved before an effective treatment is available.

Key Words: Muscle dystrophy, Gene therapy and cell therapy

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INTRODUCTION

Muscular dystrophies (MD) are a heterogenous group of hereditary muscle disorders pathologically characterized by variable fiber size, muscle fiber necrosis, phagocytosis, increased connective tissue, and regeneration[1]. In this review, we will focus on several aspects, including dystrophin defect as the Duchenne muscular dystrophy and alleic on Becker muscular dystrophy, sarcoglycan complexes deficit as the limb-girdle muscular dystrophy, merosin absence as the congenital muscular dystrophy, and fukutin defect as the Fukuyama congenital muscular dystrophy[2,3]. Clinically defined types of muscular dystrophy depend on distribution of predominant muscle weakness, age onset, clinical course, and mood of inheritance[4]. As the Duchenne muscular dystrophy is the commonest and severe form of these inherited disorders, and pathogenesis and genetic defect are well documented, we summarize the serial gene, cell and pharmacological therapies in dystrophin defect animal model and human experiences in this review.

During the past 10 years, the concept of gene therapy for muscular dystrophy has gone from an uncertain dream to a strategy moving rapidly towards clinical trials of safety. The principle of gene therapy for MD is

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simple in replacing a defective gene in the diseased muscles with the normal version of the gene. Muscle is indeed a tissue that readily expresses such transfected or transduced genes[5]. However, gene therapy is difficult to accomplish in practice. Current technology does not allow efficient distribution of genes to many muscle groups, such as different size of dystrophin gene was tried to being carried by different vectors with different efficiency, summaries in Table 1[6-12]. Numerous vectors are now available that can hold these expression cassettes and transduce muscle tissues with minimal immunological or toxic side-effects[13-16]. A major challenge to an effective treatment remains the need for an efficient systemic delivery system instead of focal muscle group injection.

MUSCULAR DYSTROPHIES

Duchenne and Becker muscular dystrophy (DMD)

The clinical feature of this disorder is characteristic of early childhood onset, proximal limbic muscle weakness with difficulty in running, later in climbing stairs[1]. Most patients have enlarged calves, which mention as pseudohypertrophy of the calves. It is also observed in patient's mother. Patients have difficulty in arising from chair or ground resulting in Gower's sign. High serum creatinine phophokinase (CK), nearly hundred time of normal subjects, however, decline in late stage by less muscle tissues remained. Some degree of mental impairment is usual, and about 20% of affected boys have an IQ of less than 70. The muscle weakness is progressive. Wheel-chair dependent would happen at the age of 12. Most DMD patients die of pneumonia or cardiac complication in second or third decade. However, a benign course exists in Becker muscular dystrophy (BMD) patients. Some BMD patients have no symptoms until much later in life, and death usually occurs in the fourth or fifth decade. X-linked inheritance with dystrophin gene mutation is well known in DMD and allelic in BMD[17]. Muscle biopsy presents as variable fiber sizes, necrotic fibers and harbors with some inflammatory reactions or phagocytosis, increased endomysial or per-

Table 1. *Different strategies in muscular dystrophy (most in Duchenne muscular dystrophy)*

Strategy	Action / effect	Advantages	Disvantages	Efficiency
Plasmid vectors	Full-length dystrophin cDNA transfer	Synthetic, non-infectious relative safe, flexible	Enhance permeability by electroporation	++
Herpes simplex	Full-length dystrophin cDNA transfer	High transduction levels in regenerating muscle, expression	Toxicity and immune response of fully function dystrophin	+
Adenoviral vectors	Full-length dystrophin cDNA transfer	High transduction levels in regenerating muscle, expression fully functional dystrophin	Toxicity and immune response, E4-coded enhancing factors assist efficiency	++
AAV vectors	Mini- or micro-truncated dystrophin cDNA	High transduction efficiencies in muscle, non-pathogenic minimal immune response	Low capacity to deliver full-length dystrophin	+++
Transgenes therapy	Alternative potential protein indepencent vector choice	Restore the function instead of the defect protein	Same as vector choice, and defect protein persistent	+
Myoblast transplantation	Introduce healthy myoblast cells	Non-infectious, relative safe	Low efficiencies, immune suppression required	+
Stem-cell therapy	Introduce dystrophin-Producing cells	Conventional treatment, relative safe	Low efficiencies, immune suppression required	+
Utrophin upregulation	Replace the function of dystrophin	More success than dystrophin transfer by vectors	Vectors transfer had toxicity and immunity, drug induce would overexpression	++

imysial connective tissues and vascularity. Sarcolemma dystrophin is usually absent in DMD muscles, but is reduced in amount or abnormal in size in BMD muscles. Definite diagnosis is depended on dystrophine gene detection in different tissue DNA extraction[18]. The dystrophin gene is by far the largest of the 30,000 genes that encodes proteins in the human genome; its 79 exons cover 2.6 million base pairs (bp). In most cases, the mutations are deletions of one or more exons (nearly 60%), duplications (6%), translocations and point mutations[19]. In general, mutations that disrupt the reading frame of the dystrophin transcript and lead to prematurely aborted dystrophin synthesis cause DMD.

Limb-girdle muscular dystrophy (LGMD)

Muscle weakness affects both males and females, with onset ranging from late in the first decade to the fourth decade. Most patients are progressive and affect primarily the pelvic and shoulder girdle muscles. So far, 15 genetically different types have been identified, which show great clinical and genetic heterogeneity[20] (Table 2). Respiratory insufficiency due to weakness of the diaphragm may occur. The distribution of weakness and rate of progression vary in intra-family members. Cardiac involvement may result in congestive heart failure or arrhythmias (type 1B, 1D, 2C, 2E, and 2F). Intellectual function remains normal. An elevated serum CK level, myopathic EMG findings, and muscle biopsy are non-specific, however, dystrophin detection is necessary for distinction of DMD/BMD features[21].

Autosomal dominant types are very rare and generally less severe than recessive type. The specific protein deficiency is known for most forms (Table 2). In LGMD2A, the defect lies in a muscle-specific, calcium-

Table 2. *Muscular dystrophies: gene location*

Disease	Mode of inheritance	Gene location	Gene product
Duchenne/Becker	XR	Xp21.2	Dystrophin
Emery-Dreifuss	XR	Xq28	Emerin
	AD	1q	lamin A & C
Facio-scapulo-humeral	AD	4q35	?
Limb-girdle			
dominant	AD	5q31	LGMD1A (myotilin)
	AD	11q-q21	LGMD1B (lamin)
	AD	3p25	LGMD1C (caveolin-3)
	AD	6q23	LGMD1D
	AD	7q	LGMD1E
Limb-girdle			
recessive	AR	15q15.1	LGMD2A (calpain-3)
	AR	2p13	LGMD2B (dysferlin)
	AR	13q12	LGMD2C (γ-sarcoglycan)
	AR	17q12-q21.33	LGMD2D (α-sarcoglycan)
	AR	4q12	LGMD2E (β-sarcoglycan)
	AR	5q33-34	LGMD2F (δ-sarcoglycan)
	AR	17q11-12	LGMD2G (telethonin)
	AR	9q31-34	LGMD2H
	AR	19q13.3	LGMD2I (fukutin related protein)
	AR	2q31	LGMD2J (titin)
Congenital MD			
Merosin deficient	AR	6q2	MDC1A (laminin α2 chain of merosin)
Fukuyama CMD	AR	9q31-33	FCMD (fukutin)
Musccle-eye-brain	AR	1p3	MEB
Walker-Warburg syndrome	AR	9q34	O-mannosynltransferase 1
Rigid spine syndrome	AR	1p36	selenoprotein N1
Ullrich syndrome	AR	21q22	collagen type VI subunite α2
Ullrich syndrome	AR	2q37	collagen type VI subunite α3

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XR: X-linked recessive; AR: autosomal recessive; AD: autosomal dominant

activated neutral protease, calpain 3. LGMD2B arises from defects in dysferlin, a novel, membrane-associated muscle protein. Four sarcoglycans (α, β, γ, δ) are deficient in LGMD2C-F.

Facioscapulohumeral muscular dystrophy (FSHD)

The incidence of this muscular dystrophy is approximately 1 in 20,000. Typical age onset is in childhood and young adulthood. Facial weakness would be the initial manifestation, and the incidence is about 50% in intrafamily affected members. It appears asymmetrical weakness in most of patients. Weakness of the shoulder girdles usually present as difficulty in elevation of their arms overhead. Scapular winging becomes apparent with attempts at abduction and forward movements of the arms. Biceps and triceps muscles may be severely affected, with relative sparing of the deltoid muscles. The involvement of heart muscles is rare, and mental impairment is not a feature. Weakness is less involved in distal part of limbs; however, weakness of the anterior compartment muscles of the legs may lead to foot drop.

The serum CK level may be normal or mildly elevated. Electormyogram (EMG) usually indicates a myopathic pattern. The muscle biopsy shows nonspecific features of a myopathy, and focal inflammatory reaction could be found in some biopsies.

This autosomal dominant inheritance pattern with almost complete penetrance has been established. FSHD is associated with subtelomeric deletion of chromosome 4q35, with loss of 3.3 kb tandem-repeat units. Loss of ten or fewer repeats causes the disorder. There is a significant correlation between disease severity and the size of the 4q35-associated deletion[22-24]. Although a specific FSH gene and protein have not been identified, and the function of gene is not clear, carrier detection and prenatal diagnosis are possible. Most sporadic cases represent new mutations[22,24].

Emery-Dreifuss muscular dystrophy (EDMD)

This disorder is childhood onset muscular dystrophy with triad of clinical features[25]. First, early contractures of elbow, Achill tendons, and posterior cervical muscles. Second, slow progressive muscle weakness and wasting with humeroperoneal muscles in distributions, then spread to the proximal limb-girdle muscles. Third, cardiomyopathy arises with conduction defects. Sudden death is not uncommon in EDMD, even in otherwise unaffected female carriers, but early use of pacemakers may be lifesaving.

There are two different inheritant transmissions in EDMD, the more common group is X-linked with defect of gene encoding emerin, which is one of nuclear membrane protein[26]. Another group is autosomal dominant inheritance, and defect of gene encoding proteins are lamin A and C, which localize to the nuclear envelope. The defect gene is located on chromosome 1[27]. The function of these proteins is not clear.

Congenital muscular dystrophies

The congenital muscular dystrophies (CMDs) comprise a heterogeneous group of muscle disorders with onset at birth or in the neonatal period[28]. Clinical features are characterized with hypotonia and weakness in floppy babies. Some forms of CMDs would have cerebral lesions with mental retardation, and the pathogenesis is stop in process of neuronal migration in the cerebral cortex[29,30]. There is a defect of some gene controls in the cortical neuron migration since embryo period resulting in lissencephaly[31]. As the genes encode to glycosyltransferases are deficient, which would cause a defect of secondary protein (α-dystroglycan), one of dystrophin-dystroglycan complex. The malfunction of dystrophin-dystroglycan complex is the clinical features of muscular dystrophyscular dystrophy[19]. Two forms without mental retardation are caused by an absence of merosin (laminin α2, a muscle extracellular protein) or very occasionally integrin α7. In cellular level, the function and relation of α-dystroglycan and merosin are well known. α-Dystroglycan is a heavily glycosylated extracellular protein that functions as a receptor for several proteins in the extracellular matrix, including laminin, agrin (a synaptic glycoprotein involved in the formation of neuromuscular junctions), the neurexins (a family of neuronal-cell-surface proteins), perlecan and biglycan[19]. The interactions between α-dystroglycan and laminin, agrin, perlecan and neurexin are dependent on dystroglycan glycosylation[32,33].

Fukuyama congenital muscular dystrophy (FCMD)

is the second only to DMD in Japan, but is seen very rarely in other populations. Onset is in infancy with hypotonia and muscle weakness. Affected children are rarely to walk. Most patients have severe mental retardation and epilepsy. Eye abnormalities are also frequently associated with FCMD[28]. Fukutin, the FCMD gene enconded protein, is correlated to this disorder; however, the true function of Fukutin is unclear. The sequence of Fukutin is similar to several putative glycosyltransferases and has an Asp-Xaa-Asp motif (where Xaa is any amino acid except proline) in its C-terminus[34]. The Asp-Xaa-Asp motif is conserved in many families of glycosyltransferases and is essential for enzymatic activity[35].

STRATEGIES TO MUSCULAR DYSTROPHY GENE THERAPY

Adenoviruses

Adenovirus vectors have a relatively large cloning capacity, easy growing in high titer and relatively efficient infection of the muscles. One of the problems, however, is the mature myofibers lack of appropriate virus attachment receptors. As these receptors are highly expressed in regenerating myofibers, recent studies to overcome this problem, using myotoxin agents induce regenerating myofibers, however, more toxin effect to host cells[36]. Alternative strategies such as vector retargeting are currently being investigated that may allow for an increase in binding of adenoviral vectors to skeletal muscle[36]. The other problem, is these vectors would elicit a reboot cellular immune response against viral and some transgene proteins. Reduced viral vector loads may help control virally mediated toxicity and immunogenicity.

Herpes simplex virus

Herpes simplex virus type-1 vectors can naturally carry large inserts. These vectors have shown relatively high transduction levels in vivo, but this is only seen in newborn and regenerating muscles. The toxicity and immunogenicity of this vector hampers the long-term expression of transgens[37].

Adeno-associated viruses (AAV)

Adeno-associated viral vectors have important advantages for gene therapy of myopathies with enough affinity for mature myofibres and small particle size (such as dysglycocan proteins and minidystrophin) facilitating the passage through the basal lamina[38,39]. However, low capacity of AAV to carry full-length protein dystrophin cDNA and using mini- or micro-dystrophin cDNA in *mdx* mouse study seem to be not as efficiency as desired. An alternative strategy to overcome this problem is using truncated dystrophin cDNA[40]. In clinic features, a large range of deletions in dystrophin cause only mild phenotypes in BMD patients, and deletions in the N-terminal domain were associated with relatively mild phenotypes. By contrast, deletions in the cysteine-rich domain cause severe dystrophy, owing to disruption of the entire dystrophin-glycoprotein complex. Harper and co-workers[41] proceeded the truncated dystrophins with different deletions in dystrophin rod domain. A 6.2 kb mini-construct (△H2-R19) that contained 8 repeats and hinge regions 1,3 and 4, was engineered to mimic the exon 17-48 deletion in a BMD patient in England. Using this mini-dystrophin cDNA was able to correct the *mdx* phenotype efficiency after AAV delivery. Another strategy to overcome low capacity of AAV is the reconstitution of the transgene by two halves coded in independent vectors[42].

Naked plasmid DNA

Plasmid displays minimal immunogenicity and toxicity, and has an extremely large cloning capacity. The disadvantage of plasmid delivery using typical delivery protocols is a relatively poor transduction efficiency. However, this method was improved by stimulating the sarcolemma (electroporation)[43]. In recent data, this method was only efficient in an animal model with local muscle group, and human clinical phase 1 trial in Paris showed no direct individual benefit for these patients[44].

Chimeroplasts

A direct method to repair or modify a mutant dystrophin gene instead of introducing healthy genes into diseased cells is with chimeroplasts, single strainded RNA/DNA oligonucleotides than can base-pair with a small target the sequence and trigger the repair of a

mutation. This approach was reported to correct the muation in *mdx* mice myoblasts in vitro, and myofibres in vivo, although with very low efficiency. Antisense oligonucleotides have also been used to influence exon/intro splicing in the dystrophin gene to either skip a mutant exon or restore an open reading frame[45,46]. While these methods are not yet efficient, but are more simple, safe, and cost-effective than viral vectors.

CELL THERAPY

Cell therapy indicates the implantation of donor cells into the affected tissues. The satellite cells are established for the source of donor myogenic cells, a potential stem cell in skeletal muscle, used to cell therapy in different muscular dystrophies, such as myoblast transplantation generates dystrophin-positive myofibres in *mdx* mice[47], merosin-positive myofibres in dy/dy mice[48], and dysferlin-positive in SJL mouse[49]. Despite some animal model observations, it is generally accepted that the efficiency is too low for the satellite cells taken from skeletal muscle limited colonize muscle tissues if delivered from the circulation. How to improve the systemical delivery and target to the diseased skeletal muscles is an important strategy for cell therapy in the future. As this reason, strategies of a cell population that could be engineered and then systemically delivered to a large number of muscles would be an invaluable tool for the development of a cell-mediated replacement therapy.

Myogenic stem cells from bone marrow

As Mavilio and his colleagues[50] found that cells derived from the bone marrow underwent myogenic differentiation and participated in muscle repair under certain muscle damage in 1998. They gave the idea of myogenic pronetitors from the bone marrow instead of satellite cells from the skeletal muscles. That study provided the first evidence that bone marrow derived stem cells were potent, and that gave the way, peripheral circulation, to transport myogenic progenitors target to the systemic skeletal muscle system.

The evidence showed differentiated muscle fibres formed by transplanted haematopoietic stem cells in models of acute or chronic muscle regeneration, including the dystrophin-deficient *mdx* mouse. The haematopoietic stem cell-to-muscle transition may be induced by certain signals provided by the regenerating muscles. Although in *mdx* mouse muscle only poorly provides the signals, even transplantation into these mice of normal C57Bl/6 bone marrow gives rise low efficiency in expressing the normal dystrophin protein (<1%) in sarcolemma. However, the ways in expansion and active recruitment to myogenic differentiation of transplanted haematopoietic cells are therefore critical factors for a future use of bone marrow transplantation in cell/gene therapy of muscular dystrophy[51].

UTROPHIN UPREGULATION

Utrophin and dystrophin are highly similar structure and biding relationships[52,53]. It is also own three-part domain as dystrophin, its c-terminal part connects to the dystrophin-glycoprotein complex[53]. In cellular level, it is initially dispersed over the sarcolemma of fetal muscles, in growing it is gradually replaced by dystrophin and only local expression in neuromuscular and myotendinous junctions of mature muscle. By contrast, in the regenerating muscles of DMD patients and *mdx* mice, utrophin was found to be both upregulated and redistributed to the sarcolemma. As this important cellular characteristic, the methods of utrophin upregulation are the possible strategies to improve and to protect cell membrane function of DMD patient and *mdx* mice. Using adenoviral delivery of mini-utrophin[54,55] or a truncated utrophin transgene[56] in animal models is also restored the dystrophin-glycoprotein complex and improved mechanical muscle performance, however, immunity and toxic effect of vectors is still concerned. Further evidence of pharmacological compounds[57] or glucocorticolids[58] upregulation utrophin, was presented that the overexpression in *mdx* mice corrects the dystrophic phenotype, and in addition, that this overexpression in non-muscular tissues is not toxic. These results indicate that utrophin upregulation need not be strictly controlled and tissue specific.

DRUG THERAPY

Many pharmacological agents have been tried in DMD[1], but they do have side effects and despite a large

number of published studies showing their efficacy, they are still not universally used. However, there still have been many trials of glucocorticoids suggested a possible slowing of the disease process, particular biopsy tissue under advanced inflammatory reaction[22,24] abstract NMD) and lower cytosolic Ca2+ levels in *mdx* myotubes resulting in less necrotic fibers[59]. Dosage and using schedule of steroid may be the point of different result of this drug strategy[26] abstract NMD). Therefore, there is a need for a Cochrane-style systemic review of published trials to make conclusion of efficiency of steroid. Albuterol, a [beta]2-agonist sympathomimetic drug, was tested in fascioscapulohumeral dystrophy[60]. Global muscle strength and function were not improved, although muscle mass and some strength measures were slightly increased. A creatine-enriched diet was useful to reduce myofibre necrosis in *mdx* mice[61].

A possible method of treating selected DMD cases (those caused by nonsense mutations) was the skipping of stop codons by aminoglycosides. Gentamicin was reported to produce good results in *mdx* mice, in which the myopathy is produced by a nonsense mutation[62]. However, other groups did not reproduce these results[63] and clinical trials have shown no positive results to date.

CONCLUSION

Many strategies to improve the function and underlying protein defects in muscular dystrophy, particular in DMD, are currently under investigation. The advantage, disadvantage, or efficiency of each strategy in the past year is summarized in Table 1. There are still have many obstacles to rescue diseased muscle such as that the effect of pharmacological trials is more convenient and understood in serious studies, but the results are still not encouraging to date. Different vectors of gene therapy are far from idea with problem to select perfect vectors from systemic delivery to target and toxicity or immunity to vectors or products. It is successful in cellular level with different strategies to regain health proteins, however, the function of muscle contraction is still a big challenge. Different approaches are focused in animal experiments towards better therapeutic strategies, but none has so far been reached the standard of a clinical test. We believe that all the problems of different approaches would be solved and can be a useful tool in a clinical help to patients with muscular dystrophies.

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