Gene Therapy for Muscular Dystrophies: Progress and Challenges

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Molecular dystrophies are groups of inherited progressive diseases of the muscle caused by mutations of diverse genes related to normal muscle function. Although there is no current effective treatment for these devastating diseases, various molecular strategies have been developed to restore the expressions of the associated defective proteins. In preclinical animal models, both viral and nonviral vectors have been shown to deliver recombinant versions of defective genes. Antisense oligonucleotides have been shown to modify the splicing mechanism of messenger ribonucleic acid to produce an internally deleted but partially functional dystrophin in an experimental model of Duchenne muscular dystrophy. In addition, chemicals can induce readthrough of the premature stop codon in nonsense mutations of the dystrophin gene. On the basis of these preclinical data, several experimental clinical trials are underway that aim to demonstrate efficacy in treating these devastating diseases.

Key Words gene therapy, muscular dystrophies, Duchenne.
edly increases the efficacy of gene transfer within muscles of an mdx mouse.6,7 In addition, the enzyme hyaluronidase, which can weaken the extracellular matrix of muscle fibers, could be used before electroporation, thus potentially further improving the efficacy of gene transfer and minimizing the physical damage to muscle fibers.8,9 However, it appears that a therapeutic application for this approach in DMD patients is unlikely.

Another method is a pressurized isolated-limb perfusion approach, which reportedly achieve a much higher efficiency than direct intramuscular injection in animal models.10 Zhang et al.11 temporarily blocked the circulation of a hindlimb in an mdx mouse with a preinjection of papaverine, and reperfused the limb with a large amount of plasmid DNA with a dystrophin gene insert. Cannulation of a large artery and the use of vasoactive agents were limitations of this approach. As an alternative, Hagstrom et al.12 demonstrated that a pressurized isolated-limb perfusion approach using a peripheral vein could also be effective in mammalian animal models. However, the transfection efficiency remained lower than that of viral vectors, even though it was markedly higher than that of intramuscular administration. Despite this, the capability of plasmid vectors to contain full-length dystrophin cDNA, as well as their noninfectious and nonimmunogenic properties, are major merits when compared to viral vectors.

In phase I study, plasmid DNA containing a full-length dystrophin gene was injected into the radialis muscle in nine patients with either DMD or Becker muscular dystrophy (BMD).13 Neither serious adverse effects nor antidystrophin immune responses were found in the study. Dystrophin was expressed in six of the nine patients, although at a low level. A clinical trial of the delivery of plasmid DNA containing a full-length dystrophin gene using a high-pressure intravascular delivery approach is currently being conducted by the Transgene and Mirus consortium.

**Viral vectors**

Over recent years, adenovirus and adenoassociated virus (AAV) have been used as viral vectors of gene therapy for DMD. Adenovirus has double-stranded DNA (35 kb); adenoviral vectors inserted with the full coding sequence of dystrophin showed efficient and functional expression of dystrophin in the mdx mouse.14 However, an acute inflammatory response and immune reactions caused by their capsid proteins limit their use as the preferred viral vector for DMD.15

The AAV is a single-stranded DNA virus (4.7 kb) and requires a helper virus such as adenovirus or herpesvirus for its replication. The major advantages of an AAV vector are its mild immune response16 and high efficiency in transducing skeletal muscles than other vectors. However, its genome is too small for the insertion of a large dystrophin gene. To overcome this problem, dystrophin microgenes were produced by removal of most of the middle rod domain and portions of the amino- and carboxyl terminals of the dystrophin gene (Fig. 1). It was reported that minidystrophin and microdystrophin could improve the phenotype of muscular atrophy in an mdx mouse model.17,18 Intravenous delivery of the AAV also demonstrated widespread transduction of cardiac and skeletal muscles in an mdx mouse model.19

In limb-girdle muscular dystrophies (LGMD), gene transfer with AAV vectors was also investigated in LGMD2D (α-sarcoglycanopathy).20 In that research, human α-sarcoglycan (α-SG) was delivered with an AAV vector via local intramuscular injection in α-SG-knockout mice. Strong and persistent expression of the α-SG gene and restoration of the dystrophin-glycoprotein complex were observed without definite cytotoxicity.

The AAV vector is currently one of the most promising viral vectors; however, there remain additional problems to be solved. Although AAV vectors seem to induce a lower immune reaction compared to adenovirus, there has been evidence of an immune response against the myofibers transduced by these vectors.21 As a possible solution to this issue, Rivière et al.22 recently reported that the use of different serotypes for the subsequent injections of AAV vectors could sustain an efficient gene transfer in skeletal muscle. In addition, the titer of AAV in animal trials has been too high, so it is necessary to reduce the dose of virus to be applied to humans. One approach is to use AAV serotypes that are more capable of crossing the vascular endothelium. Recent studies have revealed increased efficiency of gene transfer to muscle fibers with the use of newly developed serotypes.23,24 Another way is the use of agents such as vascular endothelial growth factor, which can enhance the permeability of the microvasculature to increase the extravasation of the virus.25
The clinical use of this approach was first reported by Ta-keshima et al. in 2006, when they intravenously injected ph-
osphorothioate AON aiming at exon 19 into one DMD patient weekly for 4 weeks and observed the expression of dystrophin in a muscle biopsy. However, the clinical improvement was minimal. van Deutekom et al.\textsuperscript{15} recently reported the local effects of 2OMe AON aiming at exon 51 in DMD patients, and the results were quite promising. A single dose of 0.8 mg of AON was injected into the tibialis anterior muscle in four patients with DMD. Dystrophin expression was observed in 64-97% of myofibers along with specific skipping of exon 51 in muscle biopsies performed 28 days after injection. Adverse effects were not detected. Current phase I/II trials in the United Kingdom are monitoring the local intramuscular effects of a PMO aiming at exon 51 in children with DMD (http://clinicaltrials.gov/ct2/show/NCT00159250).

**Readthrough of Stop-Codon Mutations**

A small proportion of DMD patients (about 15%) exhibit nonsense mutations. Aminoglycoside antibiotics have been known to suppress stop-codon recognition. High-dose gentamicin therapy was reported to cause readthrough of premature stop codons and allow the restoration of dystrophin expression in mdx mice.\textsuperscript{36} However, two human trials of intravenous gentamicin have failed to show a definite benefit in patients with DMD and BMD.\textsuperscript{37,38}

The readthrough efficiency of gentamicin was reported to vary markedly with different stop codons. The efficiency of translational readthrough is higher for UGA sequences than for UAG or UAA sequences. In addition, the nucleotide immediately downstream of the stop codon significantly influences the readthrough efficiency, in the order C>U>A\textsuperscript{≥}G.\textsuperscript{39}

Whilst two clinical trials have not found any toxicity associated with gentamicin, otoxicity and nephrotoxicity are well-known adverse effects associated with its long-term use. As a result, searches for new drugs that do not exhibit these adverse effects but have better readthrough efficiency have been initiated. One of those drugs, PTC124 (Ataluren, developed by PTC Therapeutics), can be taken orally and induces the readthrough of premature stop codons. Welch et al.\textsuperscript{40} reported that PTC124 restores dystrophin production in primary muscle cells from DMD patients and mdx mice. It also improves the function of striated muscle in mdx mice within 2-8 weeks of drug administration and decreases serum creatine kinase levels.

The stop-codon readthrough approach also has some disadvantages. As described previously, the readthrough efficiency of gentamicin varies with different stop codons, and chronic administration is associated with adverse effects. However, the readthrough efficiency of PTC124 does not seem vary with different stop-codons.

A phase I trial found good tolerability of PTC124 in 62 healthy adult volunteers, with only some mild adverse effects, including dizziness, headache, and gastrointestinal disturbances, being noted.\textsuperscript{41} Phase II clinical trials of PTC124 in patients with DMD and BMD are in currently in progress at multinational centers (http://clinicaltrials.gov/ct2/show/NCT-00264888, http://clinicaltrials.gov/ct2/show/NCT00592553).

**Gene Modification for Cell Therapy**

Cell therapy can be divided autologous or allogeneic. Even though genetic modification is not required in allogeneic cell therapy, the need for immunosuppression is a limitation. On the other hand, autologous cell therapy requires gene modification to restore the genetic defects. Quenneville et al.\textsuperscript{42} demonstrated the restoration of dystrophin by the cotransfection (nucleofection) of a plasmid vector containing the full-length human dystrophin gene and a phiC31 integrase in muscle precursor cells of mdx and severe combined immunodeficient (SCID) mice.

Lentivirus is currently one of the most promising viral vectors for an effective transfection of autologous cells due to its ability to incorporate into host chromosomes in various dividing cells. Lentiviral vectors expressing microdystrophin were reported to successfully transduce autologous satellite cells for transplantation into mdx mice.\textsuperscript{43} Nonmuscular-origin stem cells can also be used to restore dystrophin expression in muscles. Blood-derived CD133\textsuperscript{+} cells could be transduced with lentiviral vectors carrying a construct designed for axon skipping in dystrophic scid/mdx mice after intramuscular and intra-arterial delivery.\textsuperscript{44} In addition, Sampaolesi et al.\textsuperscript{45} demonstrated the expression of dystrophin by the transduction of mesangioblasts with a lentiviral vector containing human microdystrophin in dystrophic dogs. Their study found that muscle function and motility were also improved, together with the restoration of dystrophin.

In a phase I clinical study, autologous muscle-derived CD 133\textsuperscript{+} stem cells were transplanted into eight patients with DMD. The muscle strength did not differ significantly between treated and untreated muscles across all patients, and no local or systemic adverse events were noted.\textsuperscript{46}

**Conclusion**

Several promising strategies in gene therapy for muscular dystrophies that are currently being explored in clinical trials are reviewed herein. These strategies include gene transfer using nonviral and viral vectors, oligonucleotide-mediated exon skipping, stop-codon readthrough approaches, and gene modification for cell therapy. There remain some limitations and
challenges to be resolved in all of these strategies. Within a few years the results of various clinical trials that are currently being undertaken will become available. It is highly anticipat-
ed that at least one or two of these strategies will prove effica-
cious and evolve into an effective gene therapy for patients with DMD, BMD, and other muscular dystrophies.

Conflicts of Interest

The authors have no financial conflicts of interest.

REFERENCES

1. Acsadi G, Dickson G, Love DR, Jani A, Walsh FS, Gurusinghe A, et al. Human dystrophin expression in mdx mice after intramuscular injec-
tion of DNA constructs. Nature 1991;352:815-818.

2. Aihara H, Miyazaki J, Gene transfer into muscle by electroporation in vivo. Nat Biotechnol 1998;16:867-870.

3. Akselrod S, Viterbo E, Katzenellenbogen J, Katzenellenbogen B. High-efficiency gene transfer into skeletal muscle mediated by electric pulses. Proc Natl Acad Sci U S A 1999;96:4262-4267.

4. Durieux AC, Bonnefoy R, Bussio T, Freyssenet D. In vivo gene electrotargeting into skeletal muscle: effects of plasmid DNA on the occurrence and extent of muscle damage. J Gene Med 2004;6:809-816.

5. Molnar MJ, Gilbert R, Lu Y, Liu AB, Guo A, Larochelle N, et al. Factors influencing the efficacy, longevity, and safety of electroporation-assisted plasmid-based gene transfer into mouse muscles. Mol Ther 2004;10:447-455.

6. Vilquin JT, Kennel PF, Patoumeau-Jous M, Chapdelaine P, Boissel N, Delaere P, et al. Electrotargeting of naked DNA in the skeletal muscles of animal models of muscular dystrophies. Gene Ther 2001;8:1097-1107.

7. Murakami T, Nishi T, Kimura E, Goto T, Maeda Y, Ushio Y, et al. Full-length dystrophin cDNA transfer into skeletal muscle of adult mdx mice by electroporation. Muscle Nerve 2003;27:237-241.

8. McMahon JM, Signori E, Wells KE, Fazio VM, Wells DJ. Optimization of electroporation of plasmid into skeletal muscle by pretreatment with hyaluronidase—increased expression with reduced muscle damage. Gene Ther 2001;8:1264-1270.

9. Menmuni C, Calvaruso F, Zampaglione I, Rizzuto G, Rinaudo D, Damassa M, et al. Hyaluronidase increases electrotargeted gene transfer efficiency in skeletal muscle. Hum Gene Ther 2002;13:335-365.

10. Budker V, Zhang G, Danko J, Williams P, Wolff J. The efficient expression of intravascularly delivered DNA in rat muscle. Gene Ther 1998;5:272-276.

11. Zhang G, Ludkte J, Thioudellet C, Kleinpete P, Antoniou M, Hercejher H, et al. Intracellular delivery of naked plasmid DNA expressing full-length mouse dystrophin in the mdx mouse model of Duchenne muscular dystrophy. Hum Gene Ther 2004;15:770-782.

12. Hagstrom JE, Hegge J, Zhang G, Noble M, Budker V, Lewis DL, et al. A facile nontival method for delivering genes and siRNAs to skeletal muscle of mammalian limbs. Mol Ther 2004;10:306-308.

13. Romero ND, Braun S, Benveniste O, Leturcq F, Hogrel JY, Morris GE, et al. Phase I study of dystrophin plasmid-based gene therapy in Duchenne/Becker muscular dystrophy. Hum Gene Ther 2004;15:1065-1076.

14. DeloRusso C, Scott JM, Hartigan-O’Connor D, Salvatori G, Barjot C, Robinson AS, et al. Functional correction of adult mdx mouse muscle using gated adenoviral vectors expressing full-length dystrophin. Proc Natl Acad Sci U S A 2002;99:12979-12984.

15. Nunes FA, Farhi EE, Wilson JM, Raper SE. Gene transfer into the liver of nonhuman primates with E1-deleted recombinant adenoviral vec-
tors—safety of readministration. Hum Gene Ther 1999;10:2515-2526.

16. Jooss K, Yang Y, Fisher KJ, Wilson JM. Transduction of dendritic cells by DNA viral vectors directs the immune response to transgene prod-
ucts in muscle fibers. J Viral 1998;72:4212-4223.

17. Wang B, Li J, Xiao X. Adeno-associated virus vector carrying human minidystrophin genes effectively ameliorates muscular dystrophy in mdx mouse model. Proc Natl Acad Sci U S A 2000;97:13714-13719.

18. Yoshimura M, Sakamoto M, Ikemoto M, Mochizuki Y, Yuasa K, Miyagoe-Suzuki Y, et al. AA V vector-mediated microdystrophin expres-
sion in a relatively small percentage of mdx myofibers improved the mdx phenotype. Mol Ther 2004;10:821-828.

19. Gregorevic P, Blankinship MJ, Allen JM, Crawford RW, Meuse L, Miller DG, et al. Systemic delivery of genes to striated muscles using adeno-associated viral vectors. Nat Med 2004;10:828-834.

20. Rodino-Klapac LR, Lee JS, Mulligan RC, Clark KR, Mendell JR. Lack of toxicity of alpha-sarcoglycan overexpression supports clini-
cal gene transfer trial in LGMD2D. Neurology 2008;71:240-247.

21. Yuasa K, Sakamoto M, Miyago-Suzuki Y, Tanouchi A, Yamamoto H, Li J, et al. Adeno-associated virus vector-mediated gene transfer into dystrophin-deficient skeletal muscles evokes enhanced immune re-
sponse against the transgene product. Gene Ther 2002;9:1576-1588.

22. Rivière C, Danos O, Douar AM. Long-term expression and repeated administration of AA V type 1, 2 and 5 vectors in skeletal muscle of im-
munocompetent adult mice. Gene Ther 2006;13:1300-1308.

23. Gregorevic P, Allen JM, Minami E, Blankinship MJ, Haraguchi M, Meuse L, et al. rAA V 6-microdystrophin preserves muscle function and extends lifespan in severely dystrophic mice. Nat Med 2006;12:787-789.

24. Inagaki K, Fues S, Storm TA, Gibson GA, Metierman CF, Kay MA, et al. Robust systemic transduction with AAV9 vectors in mice: efficient global cardiac gene transfer superior to that of AAV8. Mol Ther 2006;14:45-53.

25. Mendell JR, Rodino-Klapac LR, Rosales-Quintero K, Kota J, Coley BD, Galloway G, et al. Limb-girdle muscular dystrophy type 2D gene therapy restores alpha-sarcoglycan and associated proteins. Ann Neu-
rol 2009;66:290-297.

26. Li J, Dressman D, Tao P, Sakamoto A, Hoffman EP, Xiao X. rAA V vector-mediated sarcoglycan gene transfer in a hamster model for limb girdle muscular dystrophy. Gene Ther 1999;6:74-82.

27. Monaco AP, Bertelson CJ, Liechti-Gallati S, Moser H, Kunkel LM. An explanation for the phenotypic differences between patients bearing partial deletions of the DMD locus. Genomics 1998:2:90-95.

28. Dunkley MG, Manoharan M, Villiet P, Eponer IC, Dickson G. Modifi-
cation of splicing in the dystrophin gene in cultured Mdx muscle cells by antisense oligoribonucleotides. Hum Mol Genet 1998:7:1083-1090.

29. Mann CJ, Honeyman K, Cheng AJ, Ly T, Lloyd F, Fletcher S, et al. Antisense-induced exon skipping and synthesis of dystrophin in the mdx mouse. Proc Natl Acad Sci U S A 2001;98:42-47.

30. Alter J, Lou F, Rabinowitz A, Yin H, Rosenfeld J, Wilton SD, et al. Systemic delivery of morpholino oligonucleotide restores dystrophin expression bodywide and improves dystrophic pathology. Nat Med 2006;12:175-177.

31. Lu QL, Mann CJ, Lou F, Bou-Gharious I, Morris GE, Xue SA, et al. Functional amounts of dystrophin produced by skipping the mutated exon in the mdx dystrophic mouse. Nat Med 2003;9:1009-1014.

32. Wells KE, Fletcher S, Mann CJ, Wilton SD, Wells DJ. Enhanced in vivo delivery of antisense oligonucleotides to restore dystrophin ex-
pression in adult mdx mouse muscle. FEBS Lett 2003;552:145-149.

33. Bremmer-Bout M, Aartsma-Rus A, de Meijer EJ, Kamen WE, Janson AA, Vossen RH, et al. Targeted exon skipping in transgenic hDMD mice: a model for direct preclinical screening of human-specific anti-
sense oligonucleotides. Mol Ther 2004;10:232-240.

34. Takeshima Y, Yagi M, Wada H, Ishibashi K, Nishiyama A, Kakimoto M, et al. Intravenous infusion of an antisense oligonucleotide results in exon skipping in muscle dystrophin mRNA of Duchenne muscular dystrophy. Pediatr Res 2006;59:690-694.
35. van Deutekom JC, Janson AA, Ginjaar IB, Frankhuizen WS, Aartsma-Rus A, Bremmer-Bout M, et al. Local dystrophin restoration with antisense oligonucleotide PRO051. *N Engl J Med* 2007;357:2677-2686.

36. Barton-Davis ER, Cordier L, Shoturma DI, Leland SE, Sweeney HL. Aminoglycoside antibiotics restore dystrophin function to skeletal muscles of mdx mice. *J Clin Invest* 1999;104:375-381.

37. Wagner KR, Hamed S, Hadley DW, Groisman AL, Burstein AH, Escobar DM, et al. Gentamicin treatment of Duchenne and Becker muscular dystrophy due to nonsense mutations. *Ann Neurol* 2001;49:706-711.

38. Politano L, Nigro G, Nigro V, Piluso G, Papparella S, Paciello O, et al. Gentamicin administration in Duchenne patients with premature stop codon. Preliminary results. *Acta Myol* 2003;22:15-21.

39. Howard MT, Shirts BH, Petros LM, Flanagan KM, Gesteland RF, Atkins JF. Sequence specificity of aminoglycoside-induced stop codon readthrough: potential implications for treatment of Duchenne muscular dystrophy. *Ann Neurol* 2000;48:164-169.

40. Welch EM, Barton ER, Zhuo J, Tomizawa Y, Friesen WJ, Trifillis P, et al. PTC124 targets genetic disorders caused by nonsense mutations. *Nature* 2000;400:48.164-169.

41. Hirawat S, Welch EM, Elfring GL, Northcutt VI, Paushkin S, Hwang S, et al. Safety, tolerability, and pharmacokinetics of PTC124, a non-aminoglycoside nonsense mutation suppressor, following single- and multiple-dose administration to healthy male and female adult volunteers. *J Clin Pharmacol* 2007;47:430-444.

42. Quenneville SP, Chapdelaine P, Rousseau J, Tremblay JP. Dystrophin expression in host muscle following transplantation of muscle precursor cells modified with the phiC31 integrase. *Gene Ther* 2007;14:514-522.

43. Ikemoto M, Fukuda S, Uezumi A, Masuda S, Miyoshi H, Yamamoto H, et al. Autologous transplantation of SM/C-2.6(+) satellite cells transduced with micro-dystrophin CS1 cDNA by lentiviral vector into mdx mice. *Mol Ther* 2007;15:2178-2185.

44. Benchaourir R, Meregalli M, Farini A, D’Antona G, Belicchi M, Goy-enville A, et al. Restoration of human dystrophin following transplantation of exon-skipping-engineered DMD patient stem cells into dystrophic mice. *Cell Stem Cell* 2007;1:646-657.

45. Sampaolesi M, Blot S, D’Antona G, Granger N, Tonlorenzi R, Innocenzi A, et al. Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. *Nature* 2006;444:574-579.

46. Torrente Y, Belicchi M, Marchesi C, Dantona G, Cogiamanian F, Pisat F, et al. Autologous transplantation of muscle-derived CD133+ stem cells in Duchenne muscle patients. *Cell Transplant* 2007;16:563-577.