58. Tokoyoda,K., K.Tsujikawa, Y.Ono, H.Matsushita, T.Hayashi, Y.Harada, M.Kubo, R.Abe, H.Yamamoto. Upregulation of IL-4 production by the activated cAMP/PKA pathway in CD3/CD28-stimulated naive T cells. ***International Immunology,*** 16:643-653, 2004.

＜和文＞

1. 松尾雅文、武田伸一：
最近分かった筋ジストロフィーの病態と治療.
脳と発達 38(2)： 129-131, 2006

2. 横田俊文、武田伸一：
筋ジストロフィーに対する遺伝子治療の試み.
医学のあゆみ 216(10) ： 743-747, 2006

3. 上住聡芳、鈴木直輝、武田伸一：
筋疾患の病態と診断，治療戦略の最前線
筋ジストロフィーの再生医療.
小児科診療・第 69 巻・4 号：570-574, 2006

4. 西山章代、武田伸一：
筋ジストロフィーのモデル動物と遺伝子治療.
CURRENT INSIGHTS IN Neurological Science VOL.14 No.1: 8-9, 2006

5. 大島幸子，武田伸一：
筋ジストロフィーの動物の心筋障害.
神経内科，62(6): 539-546, 2005

6. 吉村まどか，武田伸一：
筋ジストロフィーの遺伝子治療.
BRAIN MEDICAL，17(3): 221-228, 2005

7. 西山章代，武田伸一：
筋ジストロフィーのモデル動物と遺伝子治療.
Neurological Science，14(1): 8-9, 2005

8. 武田伸一，鈴木友子：
筋ジストロフィーの発症メカニズムと治療研究. -疾患解明 Overview-
実験医学，23(10): 1590-6, 2005

9. 武田伸一：
筋ジストロフィーモデル動物における遺伝子治療研究の現況と展望.
第 45 回日本神経学会総会シンポジウム，遺伝性筋ジストロフィーの根本的治療をめざして
臨床神経，44: 911-913, 2004

Ⅱ．学会発表

＜国外＞

1. Takeda S:
The NCNP dog facility
Wicker Project Workshop: Exon skipping in Muscular Dystrophy Workshop, Washington D.C., Jan 7-8, 2007

2. Takeda S:
An adeno-associated virus-mediated gene trasfer into canine X-linked muscular dystrophy in Japan ($CXMD_J$)
AFM Workshop, Evry, France, Jan 15-17, 2007

3. Takeda S：
The canine muscular dystrophy testing facility, Wellstone High Throughput Screening (HTS) Workshop
Children's National Medical Center (CNMC), Washington D.C., April 18, 2006

4. Takeda S：
Muscle stem cells and muscle regeneration
Seminar in Research Center for Genetic Medicine, Children's National Medical Center, Washington D.C., April 19,2006

5. Suzuki N, Motohashi N, Uezummi A, Fukada S, Miyagoe-Suzuki Y, Yoshimura T, Itoyama Y, Aoki M,

Takeda S:
Dislocated neuronal nitric oxide synthase controls myofiber size during tail suspension.
Vth Asian and Oceanian Myology Center Meeting in Cebu, Philippines, May 25-27, 2006

6. Ikemoto M, Fukada S, Uezumi A, Masuda S, Ampong BN, Miyoshi H, Yamamoto H, Miyagoe-Suzuki Y, Takeda S :
Transplantation of SM/c-2.6+ satellite cells transduced with micro-dystrophin CS1 cDNA by lentiviral vector into mdx mice.
American Society of Gene Therapy, Baltimore, June 1, 2006

7. Takeda S:
Gene theraphy in canine muscular dystrophy
Symposium, XIth International Congress on Neuromuscular Diseases
Istanbul, Turkey, July 3, 2006

8. Nishikawa M, Hirata K, Machida K, Takahashi Y, Yuasa K, Takeda S, Takakura Y:
Increased transgene expression of dystrophin in mdx muscle by RNAi-mediated silencing of calpain expression
American Society of Gene Therapy, Baltimore, June 1, 2006

9. Suzuki N, Motohashi N, Uezumi A, Fukada S, Miyagoe-Suzuki Y, Yoshimura T, Itoyama Y, Aoki M, Takeda S :
Dislocated neuronal nitric oxide synthase results in muscle atrophy during tail suspension
XI th International Congress of the World Muscle Society, Bruges, Belgium, October 4-7, 2006

10. Yokota T, Qi Lu, Partridge T, Nakamura A, Takeda S, Hoffman E:
Antisense morpholino injection restores extensive dystrophin expression to potentially therapeutic levels in canine muscular dystrophy in vivo
XIV Annual Congress of the European Society of Gene Therapy, Athens, Greece, November 9-12, 2006

11. Ohshima S, Shin J, Nishiyama A, Yuasa K, Nakamura A, Miyagoe-Suzuki Y, Nakai H, Takeda S:
A recombinant serotype 8 AAV-mediated gene transfer into canine skeletal muscle
XIV Annual Congress of the European Society of Gene Therapy, Athens, Greece, November 9-12, 2006

12. Nishiyama A, Ampong B, Kinoshita K, Nakai H, Takeda S:
Efficancy of adeno-associated virus serotype 8 in gene delivery into skeletal muscle of alpha-sarcoglycan deficient mice
XIV Annual Congress of the European Society of Gene Therapy, Athens, Greece, November 9-12, 2006

13. Nonaka I:
Inflammatory myopathies. At the 9th Asian and Oceanian Congress of Child Neurology. 2006; 1.26, Cebu City Philippines.

14. Fukada,S. et al. :
Molecular regulation of Quiescent Satellite Cells Revealed by Gene Expression Profiling. Frontiers in Myogenesis.2006.4, (Pine Mountain, GA, USA)

15. Takeda S:
Therapeutic approaches using micro-dystrophin and an AAV vector to dystrophin-deficient muscular dystrophy.
Seminar in Faculté des Sciences, Université de Genève, Genève,

Switzerland, Apr 14, 2005

16. Takeda S:
Muscle stem cells and muscle regeneration.
Seminar in the Biological Research Center of the Hungarian Academy of Sciences in Szeged, Hungary, Apr 19, 2005

17. Takeda S:
Therapeutic approaches using micro-dystrophin and an AAV vector to dystrophin-deficient muscular dystrophy.
Seminar in the Regional Conference Center of the Academy of Sciences in Szeged, Hungary, Apr 19, 2005

18. Takeda S:
Therapeutic approaches to dystrophin-deficient muscular dystrophy.
Seminar in the Department of Enzymology of the Hungarian Academy of Sciences in Budapest, Hungary, Apr 21, 2005

19. Takeda S:
Muscle stem cells and muscle regeneration.
Seminar in the Agricultural Research Center of Molecular Biology, Gödöllö, Hungary, Apr 21, 2005

20. Takeda S:
The Japanese approach to molecular diagnosis and therapy; special reference to ethical and legal issues.
Ethics Conference of the Hungarian Medical Chamber in Pilisszentkereszt, Hungary, Apr 22, 2005

21. Takeda S:
Participation of muscle stem cells in muscle regeneration.
EMBO/FEBS workshop "The Molecular and Cellular Mechanisms underlying Skeletal Muscle Formation and Repair", Fontevraud, France, Sep 29, 2005

22. Takeda S:
Gene therapy approach to dystrophin-deficient muscular dystrophy.
Clinical Sciences Centre Symposium in honor of Terry Partridge "From Satellite Cells to Gene Therapy", The Zoological Society of London, London, UK, Oct 1, 2005

23. Takeda S:
Contribution of CD31-negative/CD45-negative Side Population cells to skeletal muscle regeneration.
Workshop "Musculo-Skeletal: Myogenic Stem Cells and Regeneration"
8th annual meeting, American Society of Gene Therapy, St. Louis, USA. Jun 2, 2005

24. Ikemoto M, Yuasa K, Yoshimura M, Nishiyama A, Miyagoe-Suzuki Y, Howell JM, Takeda S:
An AAV vector-mediated gene transfer into canine skeletal muscle.
8th annual meeting, American Society of Gene Therapy, St. Louis, USA. Jun 2, 2005

25. Takeda S:
AAV vector mediated micro-dystrophin transfer into dytrophin-deficient skeletal muscle.
6th Japanese-French workshop on muscular dystrophies, Paris, France, July 2, 2005

26. Imamura M, Mochizuki Y, Engvall E, Takeda S:
Increased ε-sarcoglycan expression ameliorates muscular dystrophy in α-sarcoglycan deficient mice, a model for LGMD2D.
6th Japanese-French workshop on muscular dystrophies, Paris, France,

July 2, 2005

27. Nishiyama A, Yuasa K, Yoshimura M, Ohshima S, Ikemoto M, Miyagoe-Suzuki Y, Howell JM, Hijikata T, Takeda S:
AAV vector-mediated gene transfer into canine skeletal muscle.
13th Annual Congress of the European Society of Gene Therapy, Prague, Czech Ripublic, 10.30-11.1, 2005

28. Nonaka I: Special Lecture: Congenital Muscular Dystrophy. The 4th Annual Scientific Meeting of Asian & Oceanian Myology Center (AOMC), 2005.3.4, Kaohsinung, Taiwan

29. Fukada S, Uezumi A, Segawa M, Yamamoto H, Miyagoe-Suzuki Y, Takeda S:
Molecular characterization of quiescent satellite cells and their application to cell therapy
Keystone Symposia, Molecular Regulation of Stem Cells, Banff, Alberta, Canada, Feb 11, 2005

30. Uezumi A, Fukada S, Masuda S, Miyagoe-Suzuki Y, Takeda S:
Identification of a novel subpopulation of SP cells during muscle regeneration.
Keystone Symposia, Molecular Regulation of Stem Cells, Banff, Alberta, Canada, Feb 13, 2005

31. Yoshioka H, Shiga K, Takeda S, Imamura M:
In vitro analysis of assembly of the sarcoglycan complex containig ζ-sarcoglycan.
the American Society for Cell Biology, Washington DC, USA, Dec 7, 2004

32. Miyagoe-Suzuki Y, Takeda S:
Muscle stem cells as a tool for cell therapy of muscular dystrophy
Molecular Therapy of Muscular Dystrophy /Part II, International Symposium Organized by Japanese Muscular Dystrophy Research Group, Tokyo, Nov 12,2004

33. Takeda S:
The role of muscle stem cells in muscle regeneration.
Seminar at Hammer Smith Hospital, London, UK, Nov. 8, 2004

34. Takeda S:
Successful AAV vector-mediated gene transfer into canine skeletal muscle required suppression of excess immune responses.
The 12th Annual Congress of the European Society of Gene Therapy, Tampere, Finland, Nov 5, 2004

35. Takeda S:
Therapeutic approaches to dystrophin-deficient muscular dystrophy.
Seminar at Genethon, Evry, France, 7 Sep, 2004

36. Takeda S:
A novel sub-population of muscle Side Population (SP) cells and their roles in muscle regeneration.
Seminar at Pasteur Institute, Paris, France, Sep 6, 2004

37. Yoshida M, Ampong BN, Mochizuki Y, Imamura M, Takeda S:
Dysferlin may interact with dihydropyridine receptor.
9th Internatinal Congress of the World Muscle Society, Göteborg, Sweden, 2 Sep, 2004

38. Takeda S, Mochizuki Y, Uezumi A, Ojima K, Masuda S:
Contribution of bone marrow derived cells to denervated skeleral muscle.
9th Internatinal Congress of the World Muscle Society, Göteborg, Sweden, 2 Sep, 2004

39. Takeda S:
Gene transfer in Duchenne muscular dystrophy.
Monaco Round Table Discussion -Micro-dystrophin from concept to clinical trials-, Monte Carlo, Monaco, 6.19, 2004

40. Takeda S:
AAV vector and microdystrophin. Lecture at the 7th Summer School of Myology, Institut de Myologie, Paris, France, 6.17, 2004

41. Takeda S, Ikemoto M, Yoshimura M, Sakamoto M, Mochizuki Y, Yuasa K, Yokota T, Miyagoe-Suzuki Y:
An AAV vector-mediated micro-dystrophin expression in relatively small percentage of dystrophin-deficient *mdx* myofibers still improved the *mdx* phenotype through compensatory hypertrophy.
7th Annual Meeting, The American Society of Gene Therapy, Minneapolis, USA, 6.4, 2004

42. Yuasa K, Yoshimura M, Urasawa N, Sato K, Miyagoe-Suzuki Y, Howell MJ, Takeda S:
Successful AAV vector-mediated gene transfer into canine skeletal muscle required suppression of excess immune responses.
7th Annual Meeting, The American Society of Gene Therapy, Minneapolis, USA, 6.3, 2004

43. Nonaka I, Hayashi YK, Nishino I:
Recent advances in congenital muscular dystrophy research. The 4th AOMC Scientific Meeing, Kaohsiung, March 4, 2005

44. Fukada,S., et al.:
Transcriptional profiling of the high purified quiescent muscle satellite cells. Keystone Symposium (Keystone, CO, USA), 1.26, 2004.

＜国内＞

1. 武田伸一：
筋ジストロフィーに対する治療法開発東北大学医学部神経内科セミナー, 仙台市, 1.31, 2007

2. 武田伸一：
AVV ベクターを用いた筋ジストロフィーに対する遺伝子治療研究の進展
ヒトゲノム・再生医療等研究事業研究成果発表会, 東京, 2.21, 2007

3. 武田伸一：
筋ジストロフィー治療の新戦略-筋ジストロフィーに対する幹細胞移植治療
第 47 回日本神経学会総会, 東京, 5.12, 2006

4. 鈴木直輝, 武田伸一：
マウス尾部懸垂モデルにおける nNOS/NO を介した筋萎縮の分子機構の解析
第 47 回日本神経学会総会, 東京, 5.11, 2006

5. 武田伸一：
筋ジストロフィーに対する幹細胞移植治療
第27回日本炎症・再生医学会, 東京, 7.11, 2006

6. 鈴木直輝, 本橋紀夫, 上住聡芳, 深田宗一朗, 鈴木友子, 吉村哲彦, 糸山泰人,青木正志, 武田伸一：
マウス尾部懸垂モデルにおける nNOS/NO を介した筋萎縮の分子機構の解析
第27回日本炎症・再生医学会, 東京, 7.11, 2006

7. Oshima S, Nishiyama A, Yuasa K, Nakamura A, Yoshimura M, Miyagoe-Suzuki Y, Nakai H, Takeda S:
A Recombinant AAV-mediated gene

transfer into canine skeletal muscle
第 12 回日本遺伝子治療学会, 東京, 8.25, 2006

8. Nishiyama A, Beryl A.N, Yuasa K, Nakai H, Takeda S:
Efficacy of adeno-associated virus serotyoe 8 in α -sarcoglycan deficient mice
第 12 回日本遺伝子治療学会, 東京, 8.25, 2006

9. 武田伸一：
ジストロフィン欠損を巡る新たな分子病態
第 36 回小児神経学セミナー, 神奈川, 10.9, 2006

10. 武田伸一：
筋ジストロフィーに対する遺伝子治療
日本人類遺伝学会第 51 回大会, 米子市, 10.18, 2006

11. 武田伸一：
筋ジストロフィーの臨床遺伝学
第 3 回遺伝医療倫理討論ピアカウンセラー養成講座, 福岡市, 10.28-29, 2006

12. 武田伸一：
筋ジストロフィーの治療法開発の現状
藤田保健衛生大学第 4 回 21 世紀 COE 国際ワークショップ, 名古屋市, 12.5, 2006

13. 谷端淳, 鈴木直輝, 鈴木友子, 武田伸一：
内在性ユートロフィンの発現調節機構の解明
日本分子生物学会 2006 フォーラム, 名古屋市, 12.6, 2006

14. 深田総一朗, 上住聡芳, 池本 円, 増田 智, 瀬川将司, 山元 弘, 鈴木友子, 武田伸一：
骨格筋幹細胞（筋衛星細胞）の純化, 動態, 網羅的な遺伝子発現解析.
第 3 回幹細胞シンポジウム, 淡路島, 4.21, 2005

15. 鈴木友子, 武田伸一：
骨格筋幹細胞の同定とその筋再生における役割.
第 82 回日本生理学会, 仙台市, 5.20, 2005

16. 武田伸一：
最近わかった筋ジストロフィーの病態と治療「ジストロフィン欠損における新たな分子病態」
第 47 回日本小児神経学会シンポジウム, 熊本市, 5.18, 2005

17. 深田宗一朗, 上住聡芳, 池本 円, 増田 智, 瀬川将司, 山元 弘, 鈴木友子, 武田伸一：
骨格筋幹細胞（筋衛星細胞）の純化, 動態, 網羅的な遺伝子発現解析.
第 26 回日本炎症・再生医学会, 東京, 7.13, 2005

18. 鈴木直輝, 望月靖史, 上住聡芳, 深田宗一朗, 増田 智, 深瀬明子, 鈴木友子, 武田伸一：
後肢懸垂・再荷重モデルにおける筋萎縮・再成長メカニズムの解析.
第 26 回日本炎症・再生医学会, 東京, 7.13, 2005

19. 鈴木友子, 武田伸一：
骨格筋前駆細胞の維持, 増殖, 分化の分子機構：横紋筋肉腫の分子的理解に向けて.
平成 1 7 年度厚生労働省がん研究助成金森川班第 1 回班会議, 東京, 7.29, 2005

20. Yuasa K, Yoshimura M, Nishiyama A, Ikemoto M, Ohshima S, Miyagoe-Suzuki Y, McC Howell J, Hijikata T, Takeda S:
AAV vector-mediated gene transfer into canine skeletal muscle.
The 11th Annual Meeting Japan

Society of Gene Therapy, Tokyo, 7.29, 2005

21. 武田伸一：
筋ジストロフィー治療の最前線.
シンポジウム“再生医療（幹細胞移植など）の臨床的応用に関する倫理的・社会的問題”東京，9.9, 2005

22. 武田伸一：
筋ジストロフィーに対する遺伝子治療の進歩.
日本筋ジストロフィー協会施設見学会，東京，9.11, 2005

23. 武田伸一：
筋ジス治療の現状と未来.
秋田筋ジストロフィー協会シンポジウム，秋田市，9.16, 2006

24. Miyagoe-Suzuki Y, Takeda S:
Recombinant Adeno-Associated Virus (rAAV) as a therapeutic tool for Duchenne muscucular dystrophy (DMD).
“AAV and its application to Gene therapy & resenerative medicine” The 2nd Nikko International Smposium, 9.30, 2005

25. 武田伸一：
筋ジストロフィーの臨床遺伝学.
第２回遺伝医療倫理討論-ピアカウンセラー養成講座-, 名古屋市, 10.22, 2005

26. 武田伸一：
筋ジストロフィーに対する治療戦略
-遺伝子治療から薬物治療まで-
帝人研究所，1.25, 2005

27. 武田伸一：
これからの筋ジストロフィー治療.
市民公開講座第１回筋ジストロフィー　-デュシェンヌ型を中心に-
小平市，2.25, 2006

28. 武田伸一：
筋ジストロフィーに対する治療法の開発を目指して：遺伝子治療，幹細胞移植再生治療，創薬の融合的研究.
健康医療セミナー
早稲田大学科健機構，東京 2.28, 2006

29. 深田宗一朗，他，骨格筋特異的幹細胞（筋衛星細胞）の遺伝子発現解析.
第４回日本再生医療学会（大阪），3.1-2, 2005

30. 山本有希子，他，炎症性細胞機能の障害による骨格筋の再生異常の解析
骨格筋再生過程に関わる炎症細胞の機能解析，日本薬学会１２５年会（東京），3.29-31, 2005.

31. 深田総一朗，上住聡芳，瀬川雅司，増田 智，鈴木友子，山元 弘，武田伸一：
骨格筋特異的幹細胞（筋衛星細胞）の遺伝子発現解析。
第４回日本再生医療学会総会，大阪，3.1, 2005

32. 鈴木直輝，望月靖史，上住聡芳，深田総一朗，増田 智，深瀬明子，鈴木友子，武田伸一：
骨髄キメラマウスを用いた後肢懸垂・再荷重モデルにおける筋肥大メカニズムの解析。
第４回日本再生医療学会総会，大阪，3.1, 2005

33. 上住聡芳，尾嶋孝一，深田総一朗，増田智，鈴木友子，武田伸一：
骨格筋 Side Population（SP）cells の筋再生における機能。
第４回日本再生医療学会総会，大阪，3.1, 2005

34. 武田伸一：
AAV ベクターを用いた筋ジストロフィーに対する遺伝子治療の pre-clinical study － 筋ジス犬骨格筋で認められた免疫応答の克服－ 。

ヒトゲノム・再生医療等研究事業研究成果発表会，東京，2.25, 2005

35. 武田伸一：
骨格筋・幹細胞と筋再生の分子機構。
慶應大学医学部セミナー・日本横紋筋肉腫研究グループ（ＪＲＳＧ），東京，2. 14，2005

36. 武田伸一，石浦章一，鈴木友子：
内因性ユートロフィンの発現増強による筋ジストロフィーの画期的治療法の開発。
こころの健康科学（神経分野）研究成果発表会（研究者向け），東京，2.9, 2005

37. 武田伸一，鈴木友子，増田 智，深田宗一朗，鈴木直輝，望月靖史，上住聡芳：
微小重力による筋萎縮の分子メカニズム。
-メカノバイオロジーにおける分子細胞生物学的展開の最先端-，第 27 回日本分子生物学会年会，神戸市，12.10, 2004

38. 二川 健，平坂勝也，久田記美子，後藤淳平，不老治治美，大西ゆう子，岸 恭一，小川貴之，鈴江直人，安井夏生，石堂一巳，埜中征哉，武田伸二：
Unloading による筋・骨萎縮におけるユビキチン・システムの重要性ユビキチンリガーゼの結合蛋白質の解析を中心に。
-メカノバイオロジーにおける分子細胞生物学的展開の最先端-第 27 回日本分子生物学会年会，神戸市，12.10, 2004

39. 武田伸一：
骨格筋幹細胞と筋再生。
東京大学大学院セミナー，11.19, 2004

40. 武田伸一:
筋ジストロフィーに対する生殖医療と遺伝子治療。
埼玉県筋ジストロフィー協会創立 40 周念記念大会関連シンポジウム「遺伝子疾患（筋ジストロフィーなど）と生殖医療」, 9.18, 2004

41. 武田伸一：
骨格筋の幹細胞を巡る進歩。
東北大学医学部セミナー，宮城県仙台市，9.17, 2004

42. 武田伸一：
将来の治療。
平成 16 年度神経・筋疾患政策医療ネットワーク研修会，東京, 9.16, 2004

43. Ikemoto M, Yoshimura M, Sakamoto M, Mochizuki Y, Yuasa K, Yokota T, Miyagoe-Suzuki Y, Takeda S:
An AAV-vector-mediated micro-dystrophin expression in relatively small percentage of dystrophin-deficient *mdx* myofibers still improved the *mdx* phenotype.
日本遺伝子治療学会, 東京, 8.5, 2004

44. Yuasa K, Yoshimura M, Urasawa N, Sato K, Miyagoe-Suzuki Y, Howell MJ, Takeda S:
Successful AAV vector-mediated gene transfer into canine skeletal muscle required suppression of excess immune responses.
日本遺伝子治療学会, 東京, 8.5, 2004

45. 上住聡芳，尾嶋孝一，増田 智，深瀬明子，鈴木友子，武田伸一：
骨格筋再生過程における side population (SP) cells の解析。
第 25 回日本炎症・再生医学会, 東京, 7.14, 2004

46. 望月靖史，尾嶋孝一，上住聡芳，増田智，武田伸一：
骨格筋の脱神経病変に対する骨髄由来細胞の関与
第 25 回日本炎症・再生医学会, 東京, 7.13, 2004

47. 武田伸一：
筋ジストロフィーモデル動物における遺伝子治療研究の現況と展望。
三菱ウエルファーマ，横浜，5.28, 2004

48. 武田伸一：
筋ジストロフィーモデル動物における遺伝子治療研究の現況と展望。
第 45 回日本神経学会総会シンポジウム　東京　5.14, 2004

49. 吉村まどか，池本 円，坂本美喜，望月靖史，湯浅勝敏，辻 省次，武田伸二:
アデノ随伴ウイルス（AAV）ベクターによるマイクロ・ジストロフィン遺伝子の導入効果
第 45 回日本神経学会総会，東京，5.14, 2004

50. 上住聡芳，尾嶋孝一，深田宗一朗，増田 智，深瀬明子，鈴木友子，武田伸一：
骨格筋再生過程による Side Population（SP）細胞の解析
第 2 回幹細胞シンポジウム，4.26, 2004.

51. 鈴木友子：
骨格筋幹細胞と再生。獣医生理学・生化学分科シンポジウム:骨格筋研究への複合領域的アプローチ　第 137 回日本獣医学会学術集会，4.4, 2004

52. 瀬川将司，他:
骨格筋の再生過程におけるマクロファージ機能の解析，日本薬学会第 124 年会（大阪），3.29, 2004.

53. 瀬川将司，他:
骨格筋の再生過程に認められる炎症性細胞の役割，第 5 回ファーマコヘマトロジーシンポジウム（大阪），6.25, 2004.

54. 坂本健太，他:
先天性筋疾患マウスを用いた筋形成能力の解析と細胞移植療法の検討，第 54 回日本薬学会近畿支部大会（神戸），10.23, 2004.

55. 瀬川将司，他:
骨格筋再生過程に 関わる炎症細胞の機能解析，第 34 回日本免疫学会総会学術集会（札幌），12.2, 2004.

H. 知的所有権の出願・登録状況

なし

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名，巻号： ページ，出版年
Suzuki N, Miyagoe-Suzuki Y, Takeda S	Gene Therapy for Duchenne Muscular Dystrophy	***Future Neurology***, Vol 2(1), 87-96, 2007
Yugeta N, Urasawa N, Fujii Y, Yoshimura M, Yuasa K, Nakamura A, Wada M, Nakura M, Shimatsu Y, Tomohiro M,Takahashi A, Machida N, Wakao Y, Takeda S	Cardiac involvement in Beagle-based canine X-linked muscular dystrophy in Japan ($CXMD_J$): electrocardiographic, echocardiographic, and morphologic studies	***BMC Cardiovasc Disord*** 4;6:47, 2006
Uezumi A, Ojima K, Fukada S, Ikemoto M, Masuda S, Miyagoe-Suzuki Y, Takeda S:	Functional heterogeneity of side population cells in skeletal muscle	***Biochem Biophys Res Commu***, 341: 864-73, 2006
Ampong BN, Imamura M, Matsumiya T, Yoshida M, Takeda S:	Instracellulat localization of Dysferlin and its association with the Dihydropyridine receptor.	***Acta Myologica***, XXIV: 134-144, 2005
Shimatsu Y, Yoshimura M, Yuasa K, Urasawa N, Tomohiro M, Nakura M, Tanigawa M, Nakamura A, Takeda S:	Major clinical and histopathological characteristics of canine X-linked muscular dystrophy in Japan, $CXMD_J$.	***Acta Myologica***, XXIV: 145-154, 2005
Yoshimura M, Sakamoto M, Ikemoto M, Mochizuki Y, Yuasa K, Miyagoe-Suzuki Y, Takeda S:	AAV vector-mediated micro-dystrophin expression in a relatively small percentage of mdx myofibers improved the mdx phenotype.	***Mol Ther***, 10(5): 821-8, 2004

発表者氏名	論文タイトル名	発表誌名，巻号： ページ，出版年
Ojima K, Uezumi A, Miyoshi H, Masuda S, Morita Y, Fukase A, Hattori A, Nakauchi H, Miyagoe-Suzuki Y, Takeda S:	Mac-1^{low} early myeloid cells in the bone marrow-derived SP fraction migrate into injured skeletal muscle and participate in muscle regeneration.	***Biochem Biophys Res Commu***, 2004 Sep 3; 321(4): 1050-61
Fukada S, Higuchi S, Segawa M, Koda K, Yamamoto Y, Tsujikawa K, Kohama Y, Uezumi A, Imamura M, Miyagoe-Suzuki Y, Takeda S, Yamamoto H:	Purification and cell-surface marker characterization of quiescent satellite cells from murine skeletal muscle by a novel monoclonal antibody.	***Exp Cell Res,*** 2004 Jun 10; 296(2): 245-55.
Malicdan MC, Noguchi S, Nonaka I, Hayashi YK, Nishino I.	A Gne knockout mouse expressing human V572L mutation develops features similar to distal myopathy with rimmed vacuoles or hereditary inclusion body myopathy.	***Hum Mol Genet***, 16:115-28, 2007
Nishino I, Malicdan MC, Murayama K, Nonaka I, Hayashi YK, Noguchi S.	Molecular pathomechanism of distal myopathy with rimmed vacuoles.	***Acta Myol,*** 24: 80-3, 2005
Yan C, Tanaka M, Sugie K, Nobutoki T, Woo M, Murase N, Higuchi Y, Noguchi S, Nonaka I, Hayashi YK, Nishino I:	A new congenital form of X-linked autophagic vacuolar myopathy.	***Neurology***, 65: 1132-1134, 2005
Sugie K, Noguchi S, Kozuka Y, Arikawa-Hirasawa E, Tanaka M, Yan C, Saftig P, von Figura K, Hirano M, Ueno S, Nonaka I, Nishino I:	Autophagic vacuoles with sarcolemmal features delineate Danon disease and related myopathies.	***J Neuropathol Exp Neurol***, 64: 513-522, 2005
Mohri T, Fujio Y, Maeda M, Ito T, Iwakura T, Oshima Y, Uozumi Y, Segawa M, Yamamoto H, Kishimoto T, Azuma J.	Leukemia inhibitory factor induces endothelial differentiation in cardiac stem cells.	***J.Biol.Chem***, 281:6442-6447, 2006

Gene therapy for Duchenne muscular dystrophy

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Gene therapy has great potential to treat Duchenne muscular dystrophy. Among many proposed strategies to deliver a therapeutic gene to muscle, recombinant adeno-associated virus-mediated gene transfer is the most promising. The recent isolation of new adeno-associated virus serotypes from human and nonhuman primates provides the opportunity to develop vectors that can achieve the long-term expression of a therapeutic gene in muscles of the entire body without detrimental effects. To translate the results from small animal models to clinical trials in humans, further work using larger animal models, such as dystrophic dogs or nonhuman primates, is required. This review also discusses recent progress in other gene transfer-related therapeutic approaches, including targeted exon skipping and gene correction.

Duchenne muscular dystrophy (DMD), which affects one in 3300 males, is a devastating, progressive, muscle-wasting disease caused by mutations in the dystrophin gene [1,2]. Skeletal muscles in DMD are characterized by myofiber degeneration and progressive fibrous and fatty changes. There is, currently, no way to prevent muscle fiber necrosis and patients suffer severely from respiratory and cardiac complications in the second decade of life. The *DMD* gene is among the largest genes known, spanning 2.4 Mb at Xp21 and encoding a 427-kDa subsarcolemmal cytoskeletal protein, dystrophin, and several shorter isoforms (Dp260, Dp140, Dp116 and Dp71). The full-length dystrophin protein is composed of four domains: an N-terminal actin-binding domain, a central rod domain consisting of 24 spectrin-like repeats, a cysteine-rich domain and a C-terminal domain. Dystrophin binds actin at the N-terminal domain, β-dystroglycan at the cysteine-rich domain and dystrobrevin and syntrophins at the C-terminal domain, forming the dystrophin–glycoprotein complex (DGC) at the sarcolemma (Figure 1) [3]. A lack of dystrophin at the sarcolemma causes secondary loss of the DGC and other functional molecules, such as neuronal nitric oxide synthase (nNOS) [4] and aquaporin-4 [5]. Importantly, mutations in the genes encoding other members of the DGC cause several different types of muscular dystrophy. The mechanism of the degeneration and death of dystrophin-deficient myofibers is not yet fully understood, but it is believed that myofibers lacking dystrophin and the DGC at the cytoplasmic membrane are mechanically weak and highly susceptible to contraction-induced injury. As a result, the affected muscle experiences continuous cycles of myofiber death and regeneration, resulting in the gradual loss of myofibers and contractile force. In addition to mechanical weakness, abnormalities in calcium handling and changes in mitogen-activated protein (MAP) kinase and GTPase signaling in dystrophin-deficient muscle have been reported and proposed as underlying processes of muscular dystrophy [6,7].

At present, only corticosteroids are reported to effectively attenuate the progress of the disease [8], and current treatment options focus on respiratory and cardiac assistance and improvement of quality of life. Many research groups are still attempting to develop an effective therapy for DMD. In this review, we describe recent progress in gene and related therapies for DMD.

Recombinant adeno-associated virus vector: a promising tool for delivery of dystrophin gene to skeletal muscles

Among several gene transfer vectors and methods developed to date, the adeno-associated virus (AAV) vector is the most suitable to introduce the exogenous gene into postmitotic, nondividing myofibers. An AAV is a tiny, nonpathological, replication-defective virus, with a 4.7-kb single-stranded DNA genome, belonging to the parvovirus family. AAV vectors induce fewer immunological and inflammatory responses *in vivo* than adenovirus vectors [9]. Although the virus genome persists predominantly in episomal form, expression of the transferred gene lasts months to years in adult skeletal muscle. To date, more than 100 AAVs with distinct virus genome sequences have been isolated from humans, nonhuman primates and other species [10]. They display varying

Keywords: adeno-associated virus vector, Duchenne muscular dystrophy, dystrophin, exon skipping, gene therapy

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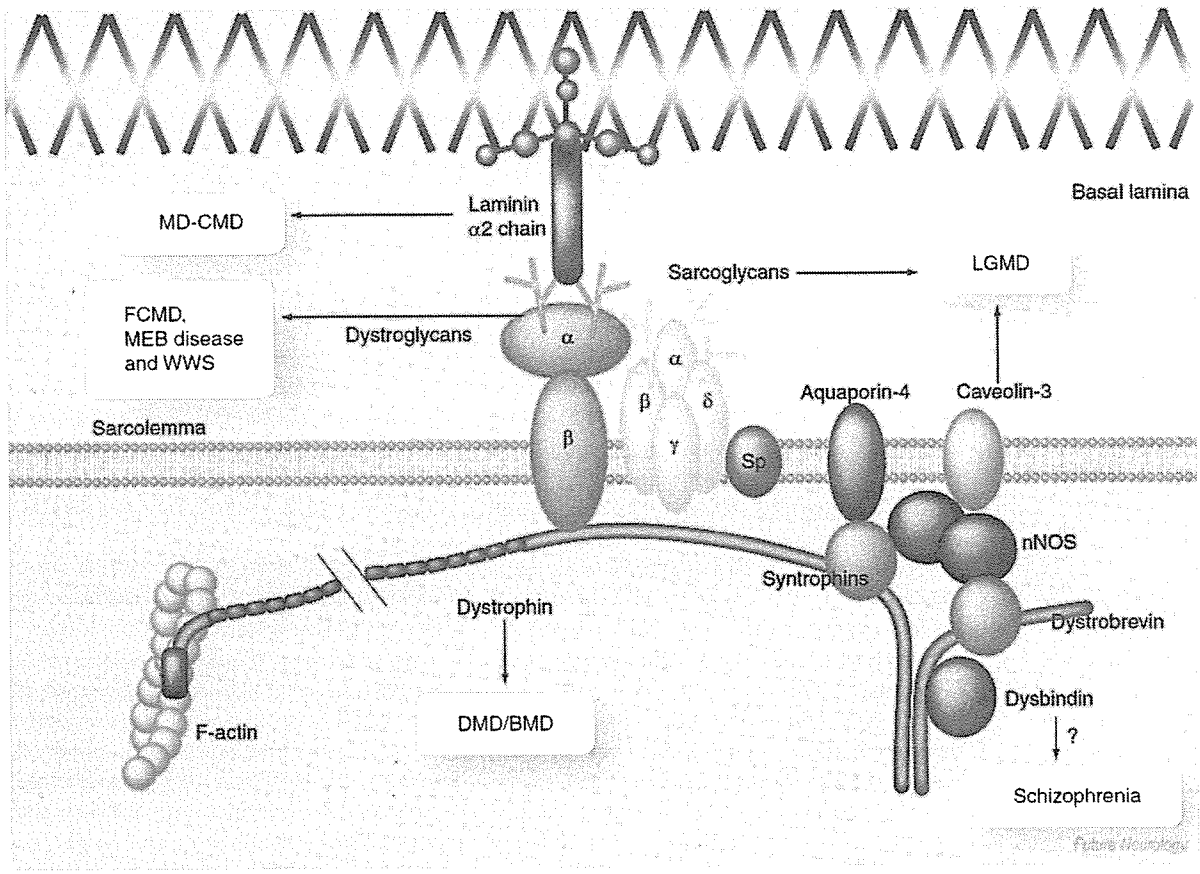
degrees of similarity in their capsid proteins and show diverse tissue tropisms. More than nine AAV vectors have already been developed and evaluated in animal models as a tool for gene transfer *in vivo* (Table 1) [10]. Although the molecular mechanisms of tissue and cell tropisms of AAV vectors are not fully explained, they are likely to use different cellular receptors for entry into and binding to the host cells. The expression of the therapeutic genes is not permanent, mainly because recombinant (r)AAV does not replicate in the host and is barely incorporated into the genome of satellite cells. They are diluted out with the turnover of myofibers and, therefore, repeated administrations are required. New AAV serotypes would provide good options for follow-up treatments because they have the potential to evade pre-existing neutralizing antibodies against the previously used AAV serotype. However, to avoid the risks of *in vivo* vector delivery, it is important to better understand the vectors and the natural infection with the corresponding virus.

Generation of microdystrophin suitable for use in rAAV vectors

The rAAV vector is a promising tool for gene transfer to DMD muscle, but the limitation of the insertion size to 4.9 kb excludes incorporation of

Figure 1. Dystrophin forms the large dystrophin–glycoprotein complex at the sarcolemma, linking the basal lamina to the cytoskeletal actin.



Mutations in the dystrophin gene end in the secondary loss of dystrophin–glycoprotein complex and other functional molecules, such as nNOS and aquaporin-4. Mutation in the laminin α2 chain gene causes congenital MD. Abnormal glycosylation of α-dystroglycan is commonly observed in FCMD, MEB disease and WWS. Abnormal glycosylation of α-dystroglycan also causes abnormalities in the eye and the CNS. Mutations in any of four sarcoglycan genes (α, β, γ and δ) result in LGMD. These observations emphasize the importance of dystrophin and associated molecules for muscle integrity. The dysbindin (*DTNBP1*) gene is one of the several putative susceptibility genes for schizophrenia.
BMD: Becker MD; DMD: Duchenne MD; FCMD: Fukuyama-type congenital MD; LGMD: Limb girdle MD; MD: Muscular dystrophy; MD-CMD: Merosin-deficient congenital MD; MEB: Muscle–eye–brain; nNOS: Neuronal nitric oxide synthase; WWS: Walker–Warburg syndrome.

Table 1. Characterization of nine serotypes of AAV vectors.

Serotype	Amino acid homology to AAV2 (%)	Isolated from	Tissue tropism				Delivery system
			Skeletal muscle	*Heart*	*Liver*	*CNS*	
1	84	NHP	+++	++	+	+	Local
2	100	Human	+	+	+	+	Local
3	88	Human		+	±	+	Local
4	64	NHP		+	±	+	Local
5	61	Human	+	+	++	++	Local
6	84	Human	++	++	+	+	Local, systemic
7	83	NHP	+++	+++	++	++	Local, systemic
8	84	NHP	+++	+++	+++	++	Local, systemic
9	83	Human	+++	+++	+++	++	Local, systemic

AAV: Adeno-associated virus; NHP: Nonhuman primate.

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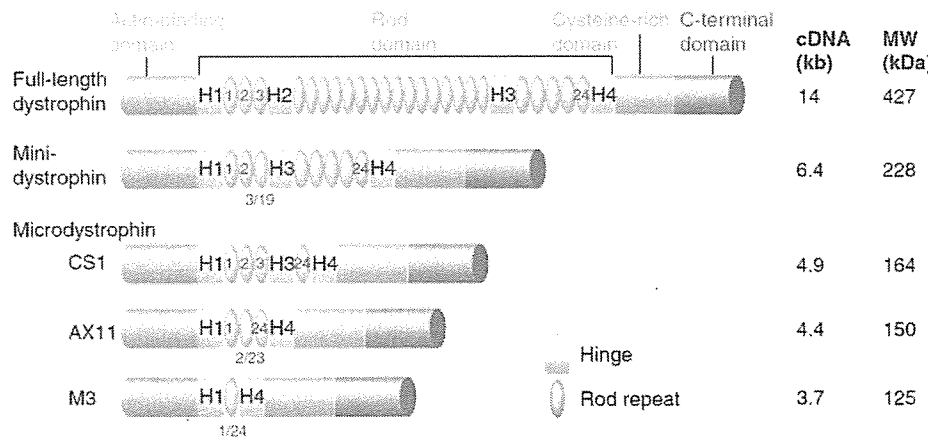
a full-length dystrophin gene (14-kb mRNA, 11-kb open reading frame). To overcome this drawback, several groups have designed small dystrophins in which the long, central rod domains are largely deleted, and tested their functions in dystrophin-deficient *mdx* mice [11]. The functions of three types of microdystrophins (CS1, AX11 and M3; Figure 2) have been tested on microdystrophin-transgenic, dystrophin-deficient *mdx* mice, and it was observed that over-expressed CS1 with four repeats and three hinges almost completely ameliorated dystrophic phenotypes [12]. Therefore, a rAAV2 vector was constructed expressing CS1, driven by a skeletal muscle-specific muscle creatine kinase promoter [13], and was injected it into the anterior tibialis muscles of immunocompetent adult *mdx* mice. A total of 24 weeks after injection, 50% of myofibers, on average, expressed microdystrophin and the treated muscles demonstrated improved contractile force [14].

Systemic delivery of rAAV-serotype 6, 8 & 9 vectors

Systemic delivery systems for the treatment of DMD require improvement to enable transfer of the therapeutic genes to the complete musculature of the body, especially to the heart and diaphragm. Gregorevic and colleagues reported that intravenous injection of rAAV6 vectors efficiently delivered a microdystrophin gene to the

Figure 2. Structure of full-length dystrophin and constructs of mini- and microdystrophin (CS1, AX11 and M3).



MW: Molecular weight.

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muscles of an adult mouse and the ratio of microdystrophin-positive fibers was increased when co-injected with vascular endothelial growth factor [15]. The widespread expression of microdystrophin was sufficient to correct susceptibility to contraction-induced injury and to lower serum creatine kinase levels [15]. Wang and colleagues, and Nakai and colleagues, demonstrated that AAV8 was more efficient than AAV6 or AAV1 at attaining systemic gene transfer, especially to the cardiac muscles of mice or hamsters, without pharmacological intervention [16,17]. More recently, Inagaki and colleagues reported that AAV9 vectors demonstrated robust systemic transduction in mice [18]. Remarkably, rAAV9 is superior to rAAV8 for gene delivery to cardiac muscle by systemic vector administration [18]. The molecular basis of the high transduction efficiency via the bloodstream is not fully understood, but these results are encouraging for researchers who are developing gene therapies for DMD patients. On the other hand, however, AAV8 or 9 vectors also increase transduction of nonmuscle tissues, such as liver (Table 1), which may be deleterious.

AAV vectors for human muscle

Animal models are indispensable for the evaluation of the efficacy and safety of AAV-mediated gene therapy of DMD, but a recent report on clinical gene transfer studies for hemophilia B demonstrated that the data obtained in preclinical studies in animals are not always predictive of vector efficacy in humans [19]. Certain human populations are exposed to AAVs in daily life: 50–96% are seropositive for AAV2 and at least a third have a neutralizing antibody to AAV2 [10]. Therefore, prior exposure to AAV2 explains the unsatisfactory results of clinical trials using rAAV2-factor IX gene transfer on hemophilia B patients [19]. The new serotypes of AAVs are reported to be prevalent in human and nonhuman primates. Prescreening of patients for neutralizing antibodies against the vector serotype and transient immune suppression would be required to avoid the elimination of rAAV particles by neutralizing antibodies.

Minidystrophin coded by two AAV vectors (dual vector system)

Microdystrophin proteins, with 3–4 spectrin-like repeats in the rod domain, do not completely compensate for the lack of full-length dystrophin. Among the constituents of DGC and its binding proteins, the expression of nNOS cannot be recovered through the introduction of microdystrophin. In an attempt to introduce a therapeutic gene larger than 4.7 kb into target cells, the trans-splicing approach, in which the gene is split between two rAAV vectors, each containing part of an intron with either a splice-acceptor or a splice-donor sequence, has been developed. After formation of head-to-tail concatamers, trans-splicing of the two RNA transcripts from the two different expression cassettes removes the intervening sequence, producing a functional mRNA larger than could be delivered in a single vector. This approach was employed to deliver a minidystrophin to *mdx* muscle [20], however, the coordinated nature of transcription and splicing makes this strategy highly inefficient *in vivo*.

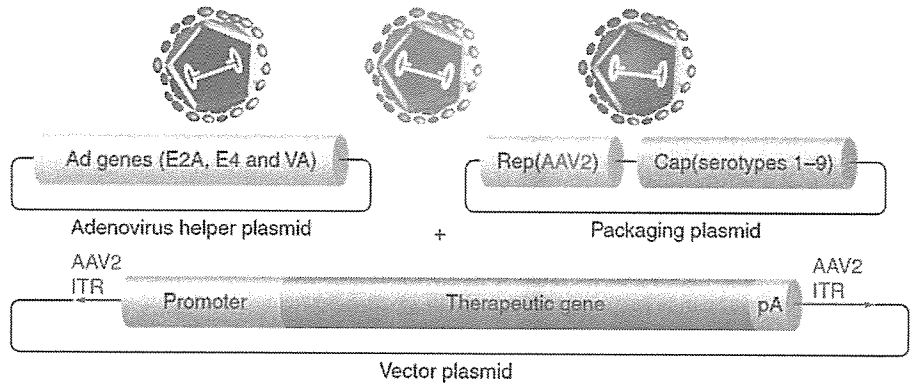
Production of AAV vectors on a large scale

rAAV vector plasmids are generated by deleting the viral genome except for the inverted terminal repeats. To obtain recombinant AAV particles, double [21] or triple [22] transfection of the plasmids into human embryonic kidney (HEK)293 cells is performed to provide rep and capsid proteins and adenoviral helper functions (Figure 3). The obtained AAV vectors are further purified by CsCl gradient sedimentation or ion-exchange chromatography. In the case of rAAV2, it is estimated that at least 1×10^{13} vg/kg is required to treat humans with hemophilia, whereas the titer of the vectors prepared by this standard method is approximately $2–5 \times 10^{13}$ genome copies from 1×10^{9} HEK293 cells [13]. In clinical trials, an inexpensive, safe, large-scale system must be developed for the production of AAV. For example, Urabe and colleagues described a highly powerful production of rAAV using nonmammalian cell culture [23]. Okada and colleagues described a large-scale AAV vector production with active gassing [24].

Safety issues

Currently, most research on AAV-mediated gene transfer focuses on the systemic delivery of therapeutic genes via the blood circulation. Some have demonstrated the effectiveness of high-pressure arterial [25] or venous [26] infusion. These procedures seem to be powerful in transducing the therapeutic genes into targeted muscle groups, but the safety should be tested carefully in larger animal models. In particular, the mutagenic and carcinogenic potentials of recombinant genomes should be investigated, in addition to their potential for germline transfer after systemic delivery.

Figure 3. Production of pseudo-typed adeno-associated virus vectors in HEK293 cells.



To obtain recombinant (r)AAV, three plasmids are transfected into human embryonic kidney (HEK)293 cells. The therapeutic gene is inserted between the two ITRs of the virus, with a ubiquitous or muscle-specific promoter. To avoid contamination of the helper adenovirus, plasmids encoding adenovirus-derived genes and *rep* and *cap* genes are cotransfected into the cells. Recently, the viral capsid gene from other AAV serotypes has been preferentially used in combination with the AAV2 vector to target the musculature body-wide *in vivo* after systemic delivery or to avoid pre-existing neutralization antibodies.
AAV: Adeno-associated virus; Cap: Capsid; ITR: Inverted terminal repeats.

Gutted adenoviral vectors expressing full-length dystrophin

Adenoviral vectors infect both dividing myoblasts and terminally differentiated muscle fibers, and possess a large insert capacity. However, early generations of adenoviral vectors, however, elicited substantial immune reactions in immunocompetent *mdx* mice and, hence, a rapid loss of transgene expression [27,28]. To circumvent this problem, a 'gutted' adenoviral vector, from which most viral DNA sequences are deleted, has been developed. Gutted adenoviral vectors are capable of carrying the large dystrophin gene together with regulatory sequences, and show reduced immunotoxicity compared with conventional adenoviral vectors [29–31]. Preparation of the gutted adenovirus vector requires a conventional adenovirus to supply replication and packaging functions *in trans*, and therefore has a high risk of helper virus contamination that may elicit immunological reactions upon delivery to tissues. In addition, recombinant adenoviral vectors remain comparatively toxic, especially in the liver, when administered systemically and have yet to achieve comparable transduction efficiency compared with AAV vectors.

Other vectors

A lentiviral vector is an alternative option for *in vivo* gene transfer into skeletal muscle. Kobinger and colleagues demonstrated that a lentiviral vector encoding minidystrophin targeted both satellite cells and myofibers of *mdx* mice and provided functional correction *in vivo* [32].

Direct injection of naked plasmid into dystrophic animals

Direct injection of a naked plasmid containing a full-length dystrophin cDNA into the muscles of DMD patients has been proposed as a promising treatment to restore the expression of dystrophin. The efficiency was low in animal models [33,34] and in a Phase I gene therapy clinical trial [35], but the dystrophin expression is relatively stable and evoked no signs of humoral or cellular immune responses. Experiments using mouse models demonstrated that the efficiency of gene transfer can be enhanced by electroporation coupled with the intramuscular application of hyaluronidase [36,37]. However, combination of electroporation and hyaluronidase administration would act to damage the muscle. The application to DMD patients is questionable. Hydrodynamic delivery of naked plasmid DNA expressing full-length dystrophin into the *mdx* mice has been reported to be effective [38]. Dystrophin expression was seen in 1–5% of the myofibers of the targeted muscle group of the hind limb for an extended period. To protect dystrophin-deficient muscles from muscle degeneration, repeated administration of plasmids would be required.

Ex vivo gene transfer into myogenic cells

Cell-mediated therapy can be used to deliver the normal dystrophin gene to dystrophic muscle. In particular, *ex vivo* transfer of a functional dystrophin gene into patients' satellite cells (myogenic progenitor cells usually located between myofibers and muscle basal lamina in a dormant state) and their progeny (myoblasts) is an attractive option for cell-based therapies for DMD since several methods to freshly purify satellite cells from muscle have been established [39,40]. A lentivirus vector would be the first choice for *ex vivo* mini- or microdystrophin gene transfer into autologous myogenic cells because it can infect freshly isolated satellite cells without lowering their proliferation and differentiation potential [Ikemoto *et al.*, Unpublished Data]. Stem cells other than satellite cells, such as muscle side population (SP) cells [41–43], mesoangioblasts [44], and AC133-positive human stem cells [43], have been reported to participate in muscle regeneration. Muscle SP cells are isolated by their ability to efflux Hoechst dye. Bachrach and colleagues demonstrated that SP cells from *mdx* (5cv) mice transduced with microdystrophin *ex vivo* were transplanted successfully via the tail vein and delivered human microdystrophin to the skeletal muscle of nonirradiated *mdx* (5cv) mice [45]. Recently, Dezawa and colleagues reported a novel method to induce muscle progenitor cells from human bone marrow stromal cells with a high efficiency [46].

Correction of endogenous genes

Gene conversion using chimeraplasts attempts to correct point mutations of the *DMD* gene in the cell. The first generation of chimeraplasts comprises hybrid RNA/DNA molecules that are homologous to a targeted gene, yet include one mismatched base. These hybrid nucleotides trigger gene conversion from a mutant to a functional allele via intranuclear DNA mismatch repair mechanisms. Injection of chimeric oligonucleotides into *mdx* mice resulted in the expression of full-length dystrophin in muscle fibers at the site of injection [47]. Gene correction mediated by chimeraplasts has also been demonstrated in the dystrophic golden retriever dog [48]. A second generation gene editing tool is a linear DNA oligonucleotide, 25-mer or longer containing a single central mismatch. This tool repaired single point mutations in the dystrophin gene with efficiencies comparable to that seen with chimeric RNA/DNA oligonucleotides, but yielded more consistent results [49]. Approximately 20% of DMD patients have single point mutations and, therefore, are potential targets of this therapeutic approach. However, gene repair techniques may not work for all mutations. Further data on the efficacy of the correction *in vivo* are required, using a range of point mutations of the dystophin gene.

Targeted exon skipping

Antisense oligonucleotides

DMD and *mdx* muscles have a few revertant fibers that express functional dystrophin [50,51]. This phenomenon is explained by aberrant splicing, which omits one or more exons and, as a result, restores a disrupted reading frame and dystrophin expression. Based on this observation, forced exon skipping is being developed as a future treatment to restore dystrophin expression from the mutated DMD gene in humans. The main tools for targeted exon skipping are antisense oligonucleotides (AOs). 2′-*O*-methyl-modified RNA on a phosphorothioate backbone, endowed oligonucleotides with greater resistance to nuclease degradation and, therefore, additional increases in stability were achieved [52–54]. Direct intramuscular injection of 2′-*O*-methyl phosphorothioate AOs resulted in a significant increase in the number of dystrophin-positive fibers (20%) in *mdx* mouse muscle [55]. Phosphoro-amide morpholino oligonucleotides have also proven to be effective in producing functional dystrophin in dystrophin-deficient muscle [56]. Weekly intravenous injections of morpholino AOs induced the expression of functional levels of dystrophin body-wide in skeletal muscles of the dystrophic *mdx* mouse and improved muscle function [57]. Based on the successful results in animal models, a clinical trial using AOs has already started in Leiden and is about to commence in the UK. Theoretically, AO-based exon skipping is applicable to 80% of dystrophin gene mutations. Furthermore, it is estimated that targeting just 12 exons restores the open reading frame of 75% of all deletions responsible for DMD.

AAV-mediated exon skipping

AOs display a limited half-life *in vivo*, and administration of AOs to patients must be repeated weekly or monthly. To obtain a longer-term effect, rAAV1 vectors expressing a modified U7 small nuclear RNA gene were used to direct exon skipping in *mdx* mice [58]. Following a single, high-pressure injection of the rAAV1/U7 vector

into the femoral artery of mdx mice, normal levels of dystrophin expression were restored and sustained for over 6 months. Although the initial study was limited to delivery to a single limb, this technique could be coupled with systemic delivery of AAV vectors of new serotypes.

Insulin-like growth factor-1 & myostatin blockade rescue dystrophin-deficient muscle

Myostatin (also known as growth and differentiation factor [GDF]8) is a transforming growth factor (TGF)-β family member that negatively regulates skeletal muscle growth, as evidenced by the increased musculature of the mice with a null mutation in this gene [59]. Mutation of the myostatin gene has also been found in human [60]. The myostatin-null child was reported to be muscular without any health problems at 4.5 years of age [60]. Myostatin blockade in *mdx* mice results in increases in both muscle mass and muscle strength and reductions in muscle fiber degeneration and serum creatine kinase levels [61]. Based on this observation, the recombinant human antibody against myostatin (MYO-029) is now being tested on adult muscular dystrophy patients.

Increased insulin-like growth factor (IGF)-1 within *mdx* myofibers reduces the breakdown of dystrophic muscle during the acute onset of muscle degeneration [62]. This mechanism of action can partly account for the long-term reduced severity of the dystrophic pathology in *mdx* mice overexpressing mIGF-1 and provides opportunities for therapeutic strategies [63].

Conclusion

Almost 20 years have passed since the discovery of dystrophin. Unfortunately, we have yet to find an effective therapy that can mitigate the dystrophic process. Numerous approaches are currently being explored, but many suffer from a variety of drawbacks. Among the gene therapy approaches to DMD under investigation, rAAV-mediated gene transfer is the most promising but still faces several obstacles. Other therapeutic approaches, including cell therapy and pharmacological intervention, would be used in complement with AAV-microdystrophin gene transfer.

Future perspective

An important step towards the clinical use of gene therapy is the evaluation of the efficacy and safety of gene transfer methods and protocols using animals larger than mice. We have established a beagle-based canine X-linked muscular dystrophy (CXMD) colony at the National Institute of Neuroscience in Japan ($CXMD_J$) and reported their severe phenotypes [64]. Beagle-based $CXMD_J$ is smaller and easier to handle than golden retriever CXMD, and is, therefore, a useful model for DMD. Preclinical studies using nonhuman primates would also be informative before clinical trials. Importantly, there are so many variables, even in a single treatment, such as myostatin blockade with antibodies, that more trials will be needed.

At present, gene therapy trials and related strategies face various hurdles and difficulties. Effective treatment of DMD may be achieved through a combination of different therapeutic approaches; for example, a combination of AAV vector-mediated gene transfer plus corticosteroid administration or myostatin blockage.

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Executive summary

Introduction

- Duchenne muscular dystrophy (DMD) is caused by mutations in the *DMD* gene, which encodes a 427-kDa subsarcolemmal cytoskeletal protein, dystrophin.
- At present, there is no treatment to arrest the progression of DMD and patients generally suffer from respiratory and/or cardiac complications in the second decade of life.
- Among several therapeutic strategies for this disease, recombinant adeno-associated virus (rAAV)-mediated gene transfer is the most promising.

Executive summary

Viral vector-mediated gene therapy

- AAV vectors drive long-term expression of the therapeutic gene in skeletal muscle *in vivo*, but the insertion size is limited to 4.9 kb.
- Functional, rod domain-deleted dystrophin (microdystrophin) can be incorporated into AAV vectors.
- New serotypes of AAV vectors have been isolated and developed as gene-transfer vectors, some of which transport the therapeutic genes to all the muscles of the body after systemic delivery.

Ex vivo *gene transfer into myogenic stem cells*

- Cell-mediated therapy can be used to deliver a normal dystrophin gene to dystrophic muscle in the hope that the delivered cells will participate in muscle-fiber regeneration in dystrophic muscle, express dystrophin and improve muscle function.
- Muscle satellite cells, side population cells, mesangioblasts, AC133-positive cells and bone marrow stromal cells are expected to be potential cell sources for cell-mediated therapy.

Gene correction & exon skipping using antisense oligonucleotides

- Chimeraplasts, which are chimeric RNA/DNA oligonucleotides homologous to a targeted gene (except for the inclusion of one mismatched base) can be used to direct the correction of a mutation by inducing preferential gene conversion from a mutant to a functional allele.
- Exon skipping using antisense oligonucleotides (AOs) targets transcribed RNA molecules to omit a nonsense mutation and restore a disrupted reading frame.
- Weekly intravenous injections of morpholino phosphorodiamidate (morpholino) AOs induce the expression of functional levels of dystrophin in skeletal muscles body-wide in the dystrophic *mdx* mouse.

Myostatin & insulin-like growth factor-1

- Blockage of myostatin and delivery of insulin-like growth factor-1 are effective to improve dystrophic phenotypes and the contractile force of dystrophin-deficient muscle.

Future perspective

- Preclinical studies using dystrophic dogs and nonhuman primates would be informative before human clinical trials.
- To overcome this devastating disease, multiple, diverse therapeutic strategies should be combined.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Koenig M, Hoffman EP, Bertelson CJ, Monaco AP, Feener C, Kunkel LM: Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals. *Cell* 50, 509–517 (1987).
2. Ray PN, Belfall B, Duff C *et al.*: Cloning of the breakpoint of an X;21 translocation associated with Duchenne muscular dystrophy. *Nature* 318, 672–675 (1985).
3. Engel AG, Ozawa E: Dystrophinopathies. In: *Myology (3rd Edition)*. Engel AG, Franzini-Armstrong C (Eds). McGraw-Hill, NY, USA, 961–1025 (2004).
   - • **Excellent text on muscular dystrophies.**
4. Brenman JE, Chao DS, Xia H, Aldape K, Bredt DS: Nitric oxide synthase complexed with dystrophin and absent from skeletal muscle sarcolemma in Duchenne muscular dystrophy *Cell* 82, 743–752 (1995).
5. Yokota T, Miyagoe Y, Hosaka Y *et al.*: Aquaporin-4 is absent at the sarcolemma and at perivascular astrocyte endofeet in α1-syntrophin knockout mice. *Proc. Japan Acad.* 76(Ser. B), 22–27 (2000).
6. Rando T: The dystrophin–glycoprotein complex, cellular signaling, and the regulation of cell survival in the muscular dystrophies. *Muscle Nerve* 24, 1575–1594 (2001).
7. Batchelor CL, Winder SJ: Sparks, signals and shock absorbers: how dystrophin loss causes muscular dystrophy. *Trends Cell. Biol.* 16, 198–205 (2006).
8. Biggar WD, Harris VA, Eliasoph L, Alman B: Long-term benefits of deflazacort treatment for boys with Duchenne muscular dystrophy in their second decade. *Neuromuscul. Disord.* 16, 249–255 (2006).
   - • **Long-term study that demonstrates that deflazacort has a very significant impact on the health and quality of life of boys with Duchenne muscular dystrophies (DMD), and is associated with few side effects.**
9. Blankinship MJ, Gregorevic P, Chamberlain JS: Gene therapy strategies for Duchenne muscular dystrophy utilizing recombinant adeno-associated virus vectors. *Mol. Ther.* 13, 241–249 (2006).
   - •• **Comprehensive review of recent progress in adeno-associated virus (AAV)-mediated gene therapy for DMD.**
10. Gao G, Vandenberghe LH, Wilson JM: New recombinant serotypes of AAV vectors. *Curr. Gene Ther.* 5, 285–297 (2005).
    - •• **Interesting review of the isolation strategy, evolution of new AAV serotypes, and their impact on gene therapy.**
11. Athanasopoulos T, Grahan IR, Foster H, Dickson G: Recombinant adeno-associated viral (rAAV) vectors as therapeutic tools for Duchenne muscular dystrophy (DMD). *Gene Ther.* 11, 109–121 (2004).
    - •• **Comprehensive review of recent progress in AAV-mediated gene therapy for DMD.**
12. Sakamoto M, Yuasa K, Yoshimura M *et al.*: Micro-dystrophin cDNA ameliorates dystrophic phenotypes when introduced into *mdx* mice as a transgene. *Biochem. Biophys. Res. Commun.* 293, 1265–1272 (2002).
13. Yuasa K, Sakamoto M, Miyagoe-Suzuki Y *et al.*: Adeno-associated virus vector-mediated gene transfer into dystrophin-deficient skeletal muscles evokes enhanced immune response against the transgene product. *Gene Ther.* 9, 1576–1588 (2002).
14. Yoshimura M, Sakamoto M, Ikemoto M *et al.*: AAV vector-mediated microdystrophin expression in a relatively small percentage of *mdx* myofibers improved the mdx phenotype. *Mol. Ther.* 10, 821–828 (2004).
15. Gregorevic P, Blankinship MJ, Allen JM *et al.*: Systemic delivery of genes to striated muscles using adeno-associated viral vectors. *Nat. Med.* 10, 828–834 (2004).

16. Wang Z, Zhu T, Qiao C *et al.*: Adeno-associated virus serotype 8 efficiently delivers genes to muscle and heart. *Nat. Biotechnol.* 23, 321–328 (2005).

• **Authors demonstrate that AAV8 vector is capable of introducing the therapeutic gene into the musculature of the whole body.**

17. Nakai H, Fuess S, Storm TA, Muramatsu S, Nara Y, Kay MA: Unrestricted hepatocyte transduction with adeno-associated virus serotype 8 vectors in mice. *J. Virol.* 79, 214–224 (2005).

18. Inagaki K, Fuess S, Storm TA *et al.*: Robust systemic transduction with AAV9 vectors in mice: efficient global cardiac gene transfer superior to that of AAV8. *Mol. Ther.* 14, 45–53 (2006).

19. Manno CS, Pierce GF, Arruda VR *et al.*: Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat. Med.* 12, 342–347 (2006).

20. Lai Y, Yue Y, Liu M *et al.*: Efficient *in vivo* gene expression by trans-splicing adeno-associated viral vectors. *Nat. Biotechnol.* 23, 1435–1439 (2005).

21. Grimm D, Kern A, Rittner K, Kleinschmidt JA: Novel tools for production and purification of recombinant adeno-associated virus vectors. *Hum. Gene Ther.* 18, 2745–2760 (1998).

22. Xiao X, Li J, Samulski RJ: Production of high-titer recombinant adeno-associated virus vectors in the absence of helper adenovirus. *J. Virol.* 72, 2224–2232 (1998).

• **One of the pioneering papers in adenovirus-free AAV preparation methods.**

23. Urabe M, Ding C, Kotin RM: Insect cells as a factory to produce adeno-associated virus type 2 vectors. *Hum. Gene Ther.* 13, 1935–1943 (2002).

24. Okada T, Nomoto T, Yoshioka T *et al.*: Large-scale production of recombinant viruses by use of a large culture vessel with active gassing. *Hum. Gene Ther.* 16, 1212–1218 (2005).

25. Gonin P, Arandel L, Van Wittenberghe L, Marais T, Perez N, Danos O: Femoral intra-arterial injection: a tool to deliver and assess recombinant AAV constructs in rodents whole hind limb. *J. Gene Med.* 7, 782–791 (2005).

26. Su LT, Gopal K, Wang Z *et al.*: Uniform scale-independent gene transfer to striated muscle after transvenular extravasation of vector. *Circulation* 112, 1780–1788 (2005).

27. Ishii A, Hagiwara Y, Saito Y *et al.*: Effective adenovirus-mediated gene expression in adult murine skeletal muscle. *Muscle Nerve* 22, 592–599 (1999).

28. Yamamoto K, Yuasa K, Miyagoe Y *et al.*: Immune response to adenovirus-delivered antigens upregulates utrophin and results in mitigation of muscle pathology in *mdx* mice. *Hum. Gene Ther.* 11, 669–680 (2000).

29. Dello Russo C, Scott JM, Hartigan-O'Connor D *et al.*: Functional correction of adult mdx mouse muscle using gutted adenoviral vectors expressing full-length dystrophin. *Proc. Natl Acad. Sci. USA* 99, 12979–12984 (2002).

30. Matecki S, Dudley RW, Divangahi M *et al.*: Therapeutic gene transfer to dystrophic diaphragm by an adenoviral vector deleted of all viral genes. *Am. J. Physiol. Lung Cell Mol. Physiol.* 287, L569–L576 (2004).

31. Dudley RW, Lu Y, Gilbert R *et al.*: Sustained improvement of muscle function one year after full-length dystrophin gene transfer into *mdx* mice by a gutted helper-dependent adenoviral vector. *Hum. Gene Ther.* 15, 145–156 (2004).

32. Kobinger GP, Louboutin JP, Barton ER, Sweeney HL, Wilson JM: Correction of the dystrophic phenotype by *in vivo* targeting of muscle progenitor cells. *Hum. Gene Ther.* 14, 1441–1449 (2003).

33. Braun S, Thioudellet C, Rodriguez P *et al.*: Immune rejection of human dystrophin following intramuscular injections of naked DNA in *mdx* mice. *Gene Ther.* 7, 1447–1457 (2000).

34. Liu F, Nishikawa M, Clemens PR, Huang L: Transfer of full-length *Dmd* to the diaphragm muscle of *Dmd* (*mdx*/*mdx*) mice through systemic administration of plasmid DNA. *Mol. Ther.* 4, 45–51 (2001).

35. Romero NB, Braun S, Benveniste O *et al.*: Phase I study of dystrophin plasmid-based gene therapy in Duchenne/Becker muscular dystrophy. *Hum. Gene Ther.* 15, 1065–1076 (2004).

36. Schertzer JD, Plant DR, Lynch GS: Optimizing plasmid-based gene transfer for investigating skeletal muscle structure and function. *Mol. Ther.* 13, 795–803 (2006).

37. Murakami T, Nishi T, Kimura E *et al.*: Full-length dystrophin cDNA transfer into skeletal muscle of adult *mdx* mice by electroporation. *Muscle Nerve* 272, 37–41 (2003).

38. Zhang G, Ludtke JJ, Thioudellet C *et al.*: Intraarterial delivery of naked plasmid DNA expressing full-length mouse dystrophin in the *mdx* mouse model of Duchenne muscular dystrophy. *Hum. Gene Ther.* 15, 770–782 (2004).

39. Montarras D, Morgan J, Collins C *et al.*: Direct isolation of satellite cells for skeletal muscle regeneration. *Science* 309(5743), 2064–2067 (2005).

40. Fukada S, Higuchi S, Segawa M *et al.*: Purification and cell-surface marker characterization of quiescent satellite cells from murine skeletal muscle by a novel monoclonal antibody. *Exp. Cell Res.* 296(2), 245–255 (2004).

41. Asakura A, Seale P, Girgis-Gabardo A, Rudnicki MA: Myogenic specification of side population cells in skeletal muscle. *J. Cell Biol.* 159, 123–134 (2002).

42. Uezumi A, Ojima K, Fukada S *et al.*: Functional heterogeneity of side population cells in skeletal muscle. *Biochem. Biophys. Res. Commun.* 341, 864–873 (2006).

43. Bachrach E, Li S, Perez AL *et al.*: Systemic delivery of human microdystrophin to regenerating mouse dystrophic muscle by muscle progenitor cells. *Proc. Natl Acad. Sci. USA* 101(10), 3581–3586 (2004).

44. Sampaolesi M, Torrente Y, Innocenzi A *et al.*: Cell therapy of α-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts. *Science* 301, 487–492 (2003).

45. Torrente Y, Belicchi M, Sampaolesi M *et al.*: Human circulating AC133$^+$ stem cells restore dystrophin expression and ameliorate function in dystrophic skeletal muscle. *J. Clin. Invest.* 114, 182–195 (2004).

46. Dezawa M, Ishikawa H, Itokazu Y *et al.*: Bone marrow stromal cells generate muscle cells and repair muscle degeneration. *Science* 309, 314–317 (2005).

47. Rando TA, Disatnik MH, Zhou LZ: Rescue of dystrophin expression in *mdx* mouse muscle by RNA/DNA oligonucleotides. *Proc. Natl Acad. Sci. USA* 97, 5363–5368 (2000).

48. Bartlett RJ, Stockinger S, Denis MM *et al.*: *In vivo* targeted repair of a point mutation in the canine dystrophin gene by a chimeric RNA/DNA oligonucleotide. *Nat. Biotechnol.* 18, 615–622 (2000).

49. Bertoni C, Morris GE, Rando TA: Strand bias in oligonucleotide-mediated dystrophin gene editing. *Hum. Mol. Genet.* 14, 221–233 (2005).