Gene Therapy Preclinical and Clinical Studies in the Treatment of Duchenne Muscular Dystrophy

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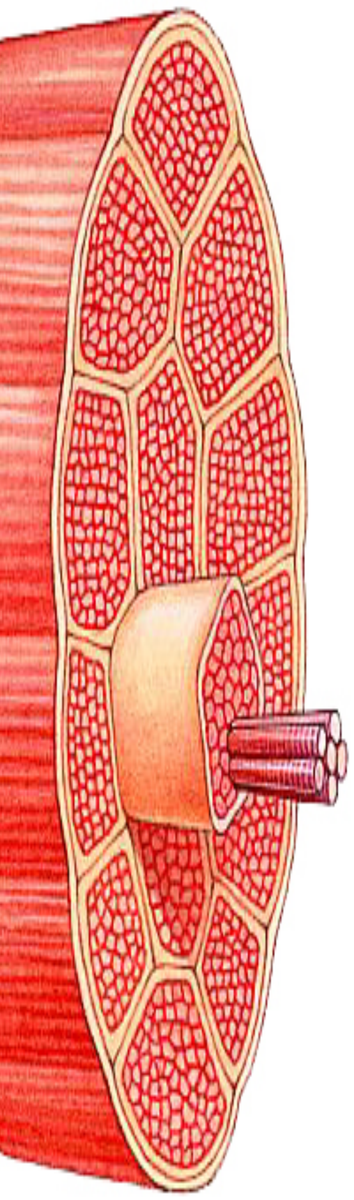

Objectives

- Understand the molecular causes of Duchenne Muscular Dystrophy (DMD) related to its characteristic symptoms.
- Provide a perspective on the gene therapy approaches as gene-replacement and gene-editing with preclincal and clinical trials.

Methodology

Collecting information from reviews and research articles published in scientific journals:

- Databases: PubMed, Scopus or Web of Knowledge.
- Impact factor: used to evaluate the relevance and quality of the journal.
- Additional information: clinical websites and DMD association videos.

Introduction

- DMD (OMIM 310200): lethal **X-linked recessive** disease, affecting 1/3500 male infants [1].
- Features: progressive muscular weakness, wasting and degeneration [1].
- Cardiac and respiratory muscles altered are life-threatening, producing a life expectancy around 30 years-old [1].
- DMD patients have mutations in DYS-gene: large deletions are the most frequent (2/3 DMD boys), followed by small mutations (insertions, deletions or substitutions) and duplications. DYS-gene, the largest gene on X-chromosome, codifies for **dystrophin (DYS**) through a 14kb mRNA [2].
- DYS (427 kDa): located in the cytoplasmic face bellow the sarcolemma. Member of the **dystrophin-associated protein complex (DAPC)** (*Fig. 1*). DYS allows both the extracellular matrix and sarcolemma to follow the contraction/relaxation produced by the contractile fibers [3].
- Lack or low presence of DYS impairs proper contraction, producing sarcolemma breaks, inflammation (Ca^{+2}), necrosis, fatty deposition and loss of muscle mass [4].
- Animal models used in preclinical trials: *mdx* and CXMD [6].

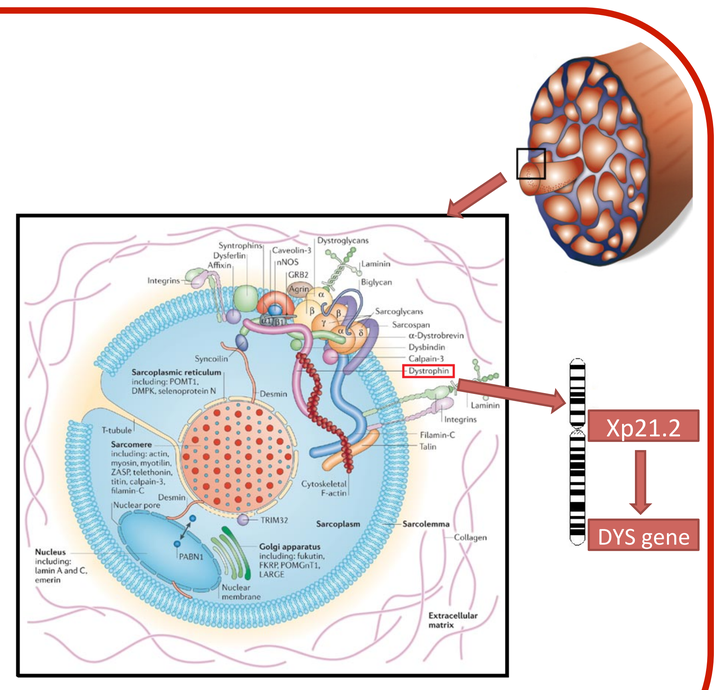

Fig. 1 The DYS situated in the sarcolemma of muscle fibers. Dystrophin interactions and Xp21.2 localization. Adapted from [3,5].

Gene Therapy

Gene-replacement

Delivery of exogenous cDNA-DYS (or surrogate genes as utrophin) into the myocytes through plasmids [7] or viral vectors [8,9].

Plasmids

- ✓ Clinical trials observed no cellular or humoral adverse effects either to injected cDNA or DYS (minimal toxicity).
- ✓ High packaging capacity allows full-length DYS delivery.
- ✓ Cheap and simple for industrial production.
- ✘ Short-term expression → Intramuscular repeated injections.

Recombinant Adeno-Associated Virus (rAAVs)

- ✓ Mainly no pathogenesis, inflammation and toxicity observed in clinical trials [10].
- ✓ Long-term and stable expression → Few intramuscular injections.
- ✓ Several serotypes (rAAV1, -6, -8, -9).
- ✘ Maximum packing capacity around 4.4 kb → synthetic minimized DYS (*Fig. 2*).
- ✘ Ab neutralization → readministrate with diverse serotype.

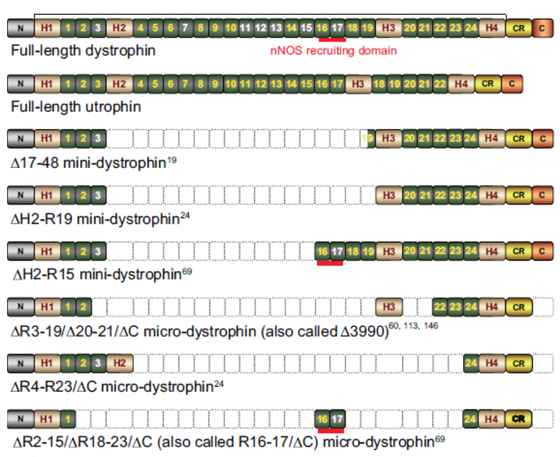

Fig. 2 Full-length DYS and diverse synthetic minimized (miniDYS and microDYS). Adapted from [6].

Gene-editing

Modification or repair of mutant DYS-gene through different methodologies tested in both preclinical and clinical trials [7-9,11].

Antisenseoligonucleotides (AON) → Exon Skipping (*Fig. 3*)

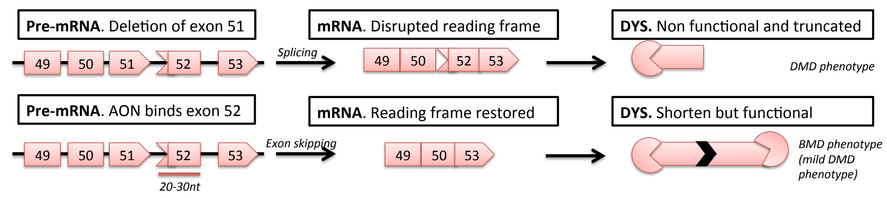

Fig. 3 Exon skipping consists in the exclusion of specific exons from the mRNA-DYS transcript during the pre-mRNA processing. The most used AON are 2'OMe and PMO: RNA residues or homologs that block splice sites or splicing regulatory regions (enhancers) of pre-mRNA.

- ✓ Clinical trials demonstrate safety, efficacy and non-toxicity.
- ✓ Cheap and simple for industrial production.
- ✘ Multiple administrations required (unestable) → rAAV
- ✘ Mutation specific and personalized → multiexon skipping targeting exons 45-55 (many patients).
- **Oligodeoxynucleotides (ODN)** are ssRNA with a non-mutated base that bind to cDNA-DYS and induce DNA-repair machinery to correct the mismatch. ODN correct single base mutations (few patients) [7].
- Read-through of premature stop codons by chemicals as **Ataluren** or **Gentamicine** enables nonsense mutation correction (few patients) [11].
- **DNA-endonucleases** (ZFNs, TALENs or MGNs) generate a double-strand break (DSB) at the cDNA-DYS desired location, which is repaired either by NHEJ or HR [11].

Conclusions

Despite all the increasing knowledge in the molecular mechanism, there is still no cure for DMD and current treatment options are limited to palliative therapy. However, new approaches in gene therapy show efficacy, safety and tolerability in clinical trials.

Future challenges: determine the optical mode of delivery for the whole muscle mass transfection, overcoming the immune response and increasing expression.

Abbreviations

2'Ome: 2'O-methyl phosphorothioate; AON: Antisense oligonucleotide; BMD: Becker muscular dystrophy; CXMD: Canine X-linked muscular dystrophy; DAPC: Dystrophin associated protein complex; DMD: Duchenne muscular dystrophy; DYS: Dystrophin; HR: Homology-directed repair; mdx: X chromosome-linked muscular dystrophy mice; MGNs: Meganucleases; NHEJ: Non-homologous end-joining; nNOS: Neuronal nitric oxide synthase; ODN: Oligodeoxynucleotides; PMO: Phosphorodiamidate morpholino oligomers; rAAV: Recombinant adeno-associated virus; TALENs: Transcription activator-like type III effector nucleases; ZFN: Zinc finger nucleases.

References

[1] Mercuri E. and Muntoni F. (2013). Muscular dystrophies. *Lancet.* 9; 381(9869):845-60.
[2] Nowak K.J. and Davies K.E. (2012). Recent advances in Duchenne muscular dystrophy. *Degenerative Neurological and Neuromuscular Disease,* 2: 141-164.
[3] Ehmsen J., Poon E. and Davies K. (2002). The dystrophin-associated protein complex. *Journal of Cell Science,* 115: 2801-2803.
[4] Blake J.D., Weir A., Newey S.E. and Davies K.E. (2002). Function and genetics of dystrophin and dystrophin-related proteins in muscle. *Physiol Rev*, 82: 291-329.
[5] Davies K.E. and Nowak K.J. (2006). Molecular mechanisms of muscular dystrophies: old and new players. *Nature Reviews Molecular Cell Biology,* 7: 762-773.
[6] Duan, D. (2011). Duchenne muscular dystrophy gene therapy: Lost in translation? *Research and Reports in Biology,* 2: 31–42.
[7] Konieczny, P., Swiderski, K. and Chamberlain, J. S. (2013). Gene and cell-mediated therapies for muscular dystrophy. *Muscle & Nerve*, 47(5): 649–63.
[8] Jarmin S. *et al.* (2014). New developments in the use of gene therapy to treat Duchenne muscular dystrophy. *Expert Opinion on Biological Therapy*, 14(2): 209–30.
[9] Goyenvalle, A., Seto, J.T., Davies, K.E., and Chamberlain, J. (2011). Therapeutic approaches to muscular dystrophy. *Human Molecular Genetics*, 20(R1): 69–78.
[10] Bowles, D. E. *et al.* (2012). Phase 1 gene therapy for Duchenne muscular dystrophy using a translational optimized AAV vector. *Molecular Therapy: The Journal of the American Society of Gene Therapy*, 20 (2): 443–55.
[11] Bertoni, C. (2014). Emerging gene editing strategies for Duchenne muscular dystrophy targeting stem cells. *Frontiers in Physiology*, 5 (148): 1-17.