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

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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 alter 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 14 kb mRNA [2].
- DYS (427 kDa): located in the cytoplasmic face below 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 (Ca²⁺), necrosis, fatty deposition and loss of muscle mass [4].
- Animal models used in preclinical trials: mdx and CXMD [6].

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.

Genes-replacement

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

Gene therapy

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

- Clinical trials demonstrate safety and non-toxicity.
- Cheap and simple for industrial production.
- Multiple administrations required (unstable) → 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 MGs) 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.

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.