
DUCHENNE MUSCULAR DYSTROPHY: REPAIR OF HUMAN MUSCLE STEM CELLS

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Can muscle stem cells from muscular dystrophy sufferers be corrected in a sustained fashion, and ultimately "cured"? Using a gene therapy technique, "exon skipping"¹, a Franco-Italian research team coordinated by CNRS researcher Luis Garcia² and Yvan Torrente³ has achieved this. These researchers have, for the first time, restored the functionality of human dystrophin, the protein that is missing in a setting of Duchenne muscular dystrophy, which is the most common of the neuromuscular diseases. They then transplanted these "corrected" human cells into mouse models of this disease in order to test their efficacy, and the muscle performance of these animals was seen to be improved. Published on December 13, 2007 in the journal *Cell Stem Cell*, this work received partial support from the Généthon, the laboratory set up and funded by the AFM thanks to donations to the Téléthon. It constitutes a step forward in autologous cell therapy using the patient's own "rehabilitated" cells.

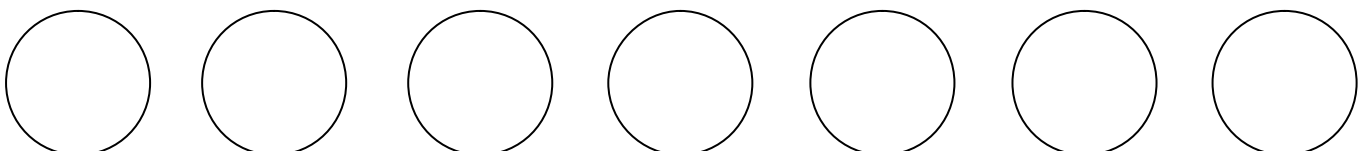
Duchenne muscular dystrophy (DMD) is a genetic disease that affects nearly one in 3500 boys at birth. It is linked to a deficit of dystrophin, a protein found under the cell membrane of muscle fibers. Coded by the DMD gene, this protein participates in the stability of muscle fibers when they are subjected to stress. In the absence of dystrophin – caused by DMD gene abnormality – it is impossible for the muscle fiber to resist the forces exerted on it during contraction, hence the gradual degeneration of the patient's muscles.

One promising lead for the treatment of certain cases of Duchenne muscular dystrophy is gene therapy by "exon skipping". This technique, which acts directly on the DMD gene message, is applied at the time of splicing, an intermediate phase between the gene and the protein. To produce a given protein, the corresponding gene delivers a message (a sort of manufacturing code) to the cell. This is made up of "coding" elements, also called exons,

¹ This technique enables restoration of the production of a protein, in this case dystrophin, which is truncated but functional.

² Since March 2007, this CNRS senior researcher has been leading the "Biothérapies des maladies neuromusculaires" team in the INSERM/Université Paris VI Joint Research Unit at the Institut de myologie.

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which need to be placed end to end. This operation is called splicing. In the case of genetic diseases, the code comprises certain abnormalities (or mutations) on one or more exons. In 65% of cases of DMD, these errors induce a shift of the message's reading frame⁴: the latter can then no longer be interpreted by the cell, thus preventing synthesis of the dystrophin protein. "Exon skipping" aims to block that part of the message comprising the abnormality (mutation) in order to restore the reading frame and allow the cell to produce the missing dystrophin in a shorter but still functional form. This technique, developed and applied in 2004 by Généthon researchers coordinated by Garcia and Olivier Danos, made it possible to restore 90% of "normal" protein in the mouse (publication in *Science*, November 2004).

More recently, a further advance has been achieved with the repair of human muscle stem cells from patients with Duchenne muscular dystrophy. The Franco-Italian research team led by Garcia (Paris) and Torrente (Milan) first of all isolated these cells, firstly from blood samples⁵, and then directly from muscle. They then "corrected" the human DMD gene mutation *in vitro* through the exon skipping technique using a system composed of a vector (lentivirus) coding for U7 small nuclear RNA⁶. The "restored" human cells were then injected into mouse models of Duchenne muscular dystrophy (via the intramuscular and intra-arterial routes). The result was that 45 days later, treated mice were expressing the human dystrophin and exhibited improved muscle performance.

This work demonstrates that it is possible to equip a patient's muscle stem cells with this system, thus enabling the sustained rehabilitation of dystrophin. This raises hope as to the possibility of autologous cell therapy⁷. Indeed, this approach can act on a patient's cell so that it "remakes" muscle. It is thus of particular interest to patients whose muscle tissue is severely damaged. Far from being in competition with other techniques (direct gene therapy by gene transfer, pharmacogenetics), the method developed by these researchers aims to be complementary and more appropriate at advanced stages of the disease, where the aim is not only to rehabilitate or restore the missing gene, but also to produce muscle once again.

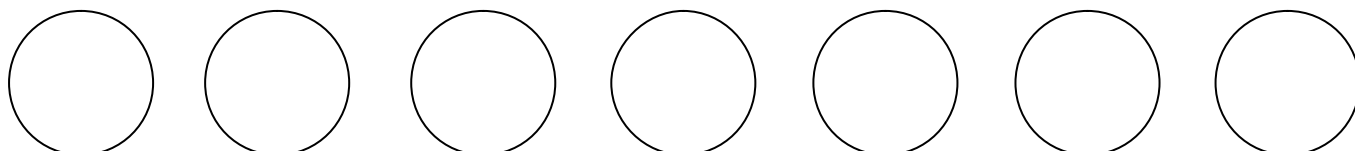
This research received financial support from the Association Française contre les Myopathies (AFM), the Association Monégasque contre les Myopathies, the Duchenne Parent Project France association, the Associazione La Nostra Famiglia Fondo DMD Gli Amici di Emanuele, the Associazione Amici del Centro Dino Ferrari, CNRS, and from Europe (MyoAMP - FP6).

⁴ The genetic code is the correspondence between triplets of nucleotides (codons) and amino acids. Its reading permits the synthesis of proteins based on genetic information. The reading frame defines which series of 3 nucleotides is read as a codon.

⁵ The main advantage of this technique is that it is less invasive than a muscle biopsy.

⁶ Ribonucleic acid

⁷ Refers to a tissue or cells obtained from a person's body and then re-administered to that person.





BIBLIOGRAPHY

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