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Pushing Therapeutic Boundaries: iPSC Therapy for Duchenne's Muscular Dystrophy

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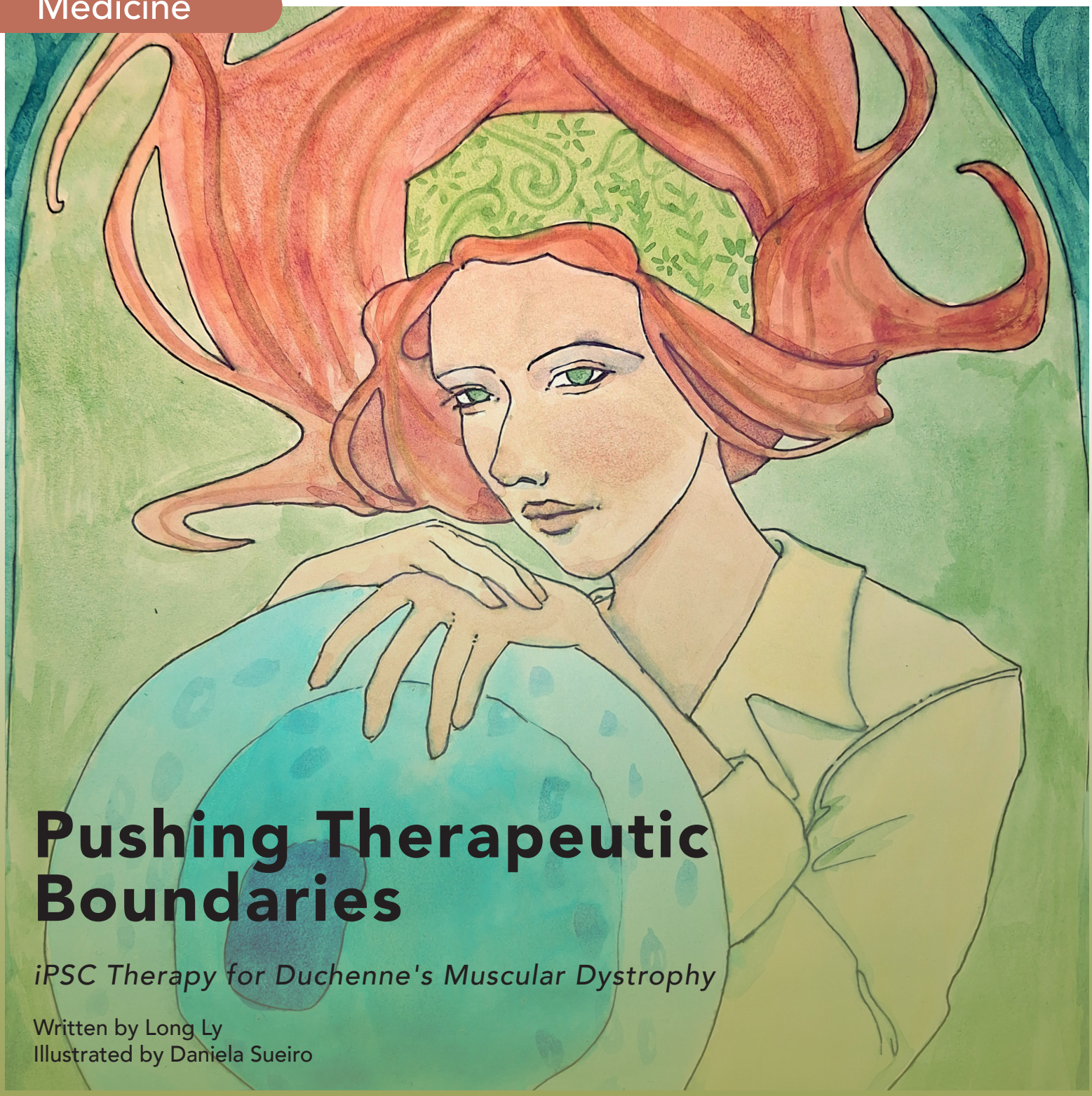

Pushing Therapeutic Boundaries

iPSC Therapy for Duchenne's Muscular Dystrophy

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Over the past few centuries, the scientific world has taken great strides toward curing disease. Doctor Edward Jenner's use of cowpox to prevent the progression of smallpox has led to the creation of vaccines. The development of the germ theory of disease by French chemist Louis Pasteur has helped us discover the origins of infectious diseases, revolutionizing many fields of medicine and vastly improving public health and sanitation. Alexander Fleming's observation of molds that make bacteria-killing substances led to the development of antibiotics, specifically penicillin, used widely to treat soldiers during World War II by curing battle wound infections and pneumonia. As our knowledge of the natural world and the human body constantly increases, so does our potential to cure disease. Nevertheless, one curse remains as the bane of medicine that we have yet to discover an adequate treatment for – genetic diseases.

Genetic disorders are often particularly challenging to treat compared to other diseases. This is because these conditions result from changes in a gene present in essentially every single cell within the body. These disorders affect several body systems and are often difficult or impossible to cure as they can only be alleviated or prolonged. One such disease is Duchenne's Muscular Dystrophy (DMD), an X-linked recessive genetic disorder characterized by significant muscle weakness and muscle atrophy.

DMD is caused by the alteration of the gene coding for dystrophin, a vital part of a protein complex that connects the cytoskeleton of muscle fibers to the extracellular matrix. The dystrophin gene is the largest gene within the entire human genome, spanning 2.6 million DNA base pairs with 79 exons. As opposed to the normal dystrophin gene, 40 percent of DMD patients have frameshift or point mutations, and 60 percent have large insertions or deletions. These cause premature truncation in translation, making the dystrophin protein unstable and non-functional. DMD patients exhibit their earliest symptoms at two to three years of age, some of which may include difficulty climbing stairs and fumbling. The progression of DMD causes patients to be wheelchair-bound as early as 12 years old and require assisted ventilation by 20. Even with optimal medical treatment, patients only survive until 20 or 40 years old, with the primary causes of death being respiratory or heart failure. The severity of DMD and patients' low quality of life demand treatment to alleviate these symptoms.

Currently, there are a few known methods used to treat DMD. Glucocorticoids have been used to slow the progression of DMD and increase muscle strength. Gene replacement therapy introduces the correct form of a gene through a viral vector, which inserts a gene inside a cell to override the faulty gene. The effectiveness depends on whether there is long-term delivery of the correct gene and persistent gene correction in most muscle fibers in DMD patients. Intramuscular injections only transduce cells a few centimeters from the injection site, which means that most injections will have to be done throughout the body, including the heart muscle, making the treatment difficult and improbable in certain areas. Another therapy comes from the observation that at the early stages of DMD, the existing myoblasts in our body can fuse together to form new muscle fibers. However, that proliferative potential is eventually exhausted. Myoblast transplantation, the delivery of myogenic cells, is therefore used to combat muscle atrophy in DMD patients. Mutation-specific therapies have also been used to treat DMD since DMD mutations can be nonsense, missense, deletion, or duplication. Around five to 15 percent of mutations are caused by nonsense mutations, which result in an early stop codon. These are usually more detrimental to patients because they generate an incomplete protein that is often non-functional, especially if the stop codon occurs early in the dystrophin gene. Thus, drugs that promote translational readthrough of stop codons, such as aminoglycoside antibiotics, have been suggested. They produced hopeful results in mouse models of DMD but did not perform in human trials. These are among the many therapies currently being investigated or used to treat DMD, and many have shown promise. However, there is currently no permanent, sufficiently effective, and inexpensive treatment to combat muscle wasting in DMD.

This therapy removes the ethical issues involved in using embryonic stem cells for stem cell therapy and exceeds the regenerative potential of adult stem cells or constant myoblast transplantation.

A novel treatment being discussed to combat DMD is patient-derived induced pluripotent stem cells (iPSCs). The therapy involves several steps. By forcing the expression of four embryonic transcription factors, OCT4, SOX2, KLF4/MYC, and NANOG/LIN28, iPSCs can first be generated from adult somatic cells of the patient, like fibroblasts. Following the induction of pluripotency, genome editing technologies such as TALEN or CRISPR-Cas9 are used to correct the dystrophin gene. The iPSCs are then cultured in large quantities, induced to differentiate into myogenic progenitors and precursor cells, and subsequently transplanted back into the patient. Patient-derived iPSCs therapy combines several past ideas, such as the usage of cells with regenerative capabilities, genome editing to introduce functional copies of dystrophin, and transplantation of stem cells into the patient. Currently, patient-derived iPSC therapy is limited to mouse models of X chromosome-linked muscular dystrophy (mdx) but shows promise in increasing muscle regeneration and functional recovery.

Patient-derived iPSC therapy provides several potential benefits and solves certain problems that other therapies may have. This therapy removes the ethical issues involved in using embryonic stem cells for stem cell therapy and exceeds the regenerative potential of adult stem cells or constant myoblast transplantation. Due to the aforementioned increased regenerative capabilities, it concurrently removes some of the need to supply new myoblasts from donors consistently.. Patient-derived iPSC therapy also addresses an important issue with using donor iPSCs. When donor iPSCs are transplanted into the patient, it may cause immune rejection due to a mismatch in Human Leukocyte Antigen proteins, the human version of Major Histocompatibility Complex (MHC) at the cell membrane. Using patient-derived genome-edited iPSCs removes this issue for obvious reasons.

However, there are certain downfalls that patient iPSC therapy presents. Although iPSC cells have commonly been generated using fibroblasts, some studies have reported that iPSCs retain some epigenetic memory from the somatic cells that may result in limited differentiation potential for certain cell types, including muscle progenitors. It is also noted that some residual epigenetic memory diminishes after some time in cell culture. Another challenge is finding the optimal method of delivery of the embryonic transcription factors to derive iPSCs. There have been concerns about the fact that certain delivery methods integrate these transcription factors into the genome, which would inactivate the tumor suppressor genes that are significant in the development of cancer. Approaches that use transient and integration-free methods are preferred for deriving clinically safe iPSCs. Another concern comes from the fact that iPSCs can accumulate chromosomal abnormalities, genetic instability, and loss of heterozygosity among other genetic alterations. There is still a need to study and refine our methods to develop a safer and more effective therapy.

The discovery of generating iPSCs has been revolutionary in the field of regenerative medicine. It has opened the doors to possible treatments for many previously incurable and debilitating diseases and disorders. Although iPSC therapy requires a lot more experimentation, discovery, and refinement, it is an intriguing as well as promising approach to treating disease.

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