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Duchenne's Muscular Dystrophy

Research is a dynamic enterprise that generates a wealth of knowledge. It provides a forum for debating ideas and working them into evidence-based theories. The clinical trial setting puts these theories to the test and may lead to evidence-based medicines that can alleviate symptoms or cure disease. But the process of taking research from bench to bedside is a lengthy one, and demands not only vision but also years of hard work and dedication on the part of scientists, physicians and patients. This document presents some basic information about Duchenne's muscular dystrophy and, in so doing, frames the context for the discussion that follows about the future application of stem cells to treat this disease. The content presented is by no means exhaustive and readers may wish to peruse additional web resources or speak with their physicians for more information.

About Muscular Dystrophy

Muscular dystrophies are a group of inherited neuromuscular disorders that lead to progressive muscle weakness and wasting. The most common and severe form of the disease is Duchenne's muscular dystrophy. It afflicts one in every 3,500 boys who typically succumb to paralysis and death in their twenties from respiratory or cardiac complications. Other types of muscular dystrophies are less debilitating than Duchenne's and can affect both males and females at various ages.

Causes

Duchenne's muscular dystrophy (DMD) is caused by a mutation in the dystrophin gene, the largest known gene, located on the X chromosome. Dystrophin is an anchor-type protein that sits just inside the muscle cell membrane and tethers structural proteins inside the cell to the proteins that sit in the outer surface of the cell membrane. This connectivity effectively stabilizes the muscle cells, which are bundled together to form muscles. The most common type of mutation in the dystrophin gene is a deletion where part of the gene is missing but duplications and single point mutations can also occur. As a result, the body cannot make dystrophin, a protein vital for keeping muscle cells intact during the normal wear and tear of the day, and muscle cells are very susceptible to damage. Under normal conditions, damaged muscles try to regenerate. But without dystrophin, the normal pattern of degeneration and regeneration is compromised. At a certain point, the pool of stem cells that make new muscle become exhausted, and more degeneration than regeneration occurs. As a result, the muscle becomes inflamed, is replaced with fibrous and fatty tissue, and simply wastes away over time.

An X-linked disease

Duchenne's muscular dystrophy is commonly referred to as an X-linked disease. This means that, in the majority of cases, the mutation in the dystrophin gene is inherited from the mother. The gene for dystrophin is located only on the X chromosome, one of the two sex chromosomes (the other is the Y chromosome). Females have two copies of the X chromosome and therefore have two copies of the dystrophin gene. It is rare for girls to succumb to an X-linked disease because if there is a defective gene on one X chromosome, then the same gene on the other X chromosome can usually compensate. In contrast, boys have only one X chromosome, inherited from the mother, and one Y chromosome from the father. The Y chromosome does not carry a dystrophin gene so there is no way of compensating if

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the dystrophin gene on the X chromosome is defective.

Symptoms and diagnosis

Duchenne's muscular dystrophy is usually detected between the ages of three and five when parents first notice that a child has difficulty walking, falls frequently or cannot keep up with children of the same age. As more muscles become compromised by the disease children develop difficulty breathing, talking, or holding themselves upright. By the age of eight to ten boys often require braces, and by age 12 they are usually confined to a wheelchair. If untreated, boys with Duchenne's rarely live into their twenties or thirties.

Three tests are commonly used to confirm a diagnosis of Duchenne's. Physicians will measure the levels of an enzyme called CK which, when present in high amounts, signal that muscle cells are breaking down. Mutations in the dystrophin gene are detected through genetic testing, and muscle biopsies will reveal whether dystrophin is lacking in the muscles.

Treatment

There are no current therapies that cure Duchenne's muscular dystrophy. The mainstay of treatment includes steroids, physiotherapy, braces and wheelchairs, spinal surgery, and breathing aids. These treatments help to improve the patient's quality of life by controlling symptoms, strengthening muscles, continuing mobility and dealing with the ongoing challenges of the illness. Steroids, such as prednisone, remain the only treatment that is capable of slowing the progression of disease, but even so only for short periods of time (up to three years) and not without considerable side effects, including weight gain and increased risk of fractures.

New therapies to treat Duchenne's muscular dystrophy are sorely needed. One of the biggest challenges for treating this disease is how to deliver therapies when the target tissue is the entire mass of skeletal muscle distributed throughout the body. In the case of gene therapy, one of the main goals is to find vectors suitable for delivering a gene as big as dystrophin. As the field advances on all fronts, there are newer more promising therapies being developed which focus on drugs that ameliorate the symptoms of DMD, and gene therapies that correct the dystrophin defect or rescue the unaffected part of the DMD gene that can still make a dystrophin protein. Cell-based therapies aim to replace the dystrophin protein or the cells that make it, either through using the cells themselves as vectors to deliver therapies or for their inherent regenerative properties.

The cell-based approach, in particular as it applies to stem cells, is the focus for the remainder of the material presented here on Duchenne's muscular dystrophy.

Can Stem Cells Help?

Because Duchenne's muscular dystrophy is caused by the deficiency of one gene product – the protein dystrophin – scientists are hopeful that stem cells will someday be viable options for treating this disease. As things stand, the two main applications of stem cells for treating Duchenne's are as vehicles for delivering gene therapies that replace or repair the defective DMD gene, and/or as cells that regenerate damaged muscle.

Scientists have identified different stem cells that are myogenic or able to form muscle. They can be found in skeletal muscle, bone marrow, blood, fat, and other tissues. Although figuring out which stem cells are ideal for treating Duchenne's is no mean feat, there are certain qualities that make some stem cells better candidates than others.

Scientists must be able to grow and expand stem cells in the laboratory under conditions that maintain the 'stemness' of the cells – that is the ability to differentiate into muscle cells and self-renew to maintain the muscle-forming stem cell pool. Ideally, the stem cell of choice should be amenable to a system-wide delivery technique given that skeletal muscle throughout the body can be affected in Duchenne's. Early experiments which transplanted muscle-forming cells by intramuscular injection were foiled because the spread of the cells was far too limited to be of any benefit. Since then, researchers have deduced that the circulatory system is a better delivery route because the transplanted cells can be shuttled throughout the body via the blood.

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Of course, once stem cells are transplanted, they must be able to survive, grow and migrate to the site of damaged skeletal muscle. If at all possible, the stem cell graft should be able to do this without eliciting an immune response that would lead to graft rejection in the recipient. As well as being able to repair or rebuild muscle fibres, the best stem cell candidate should also be able to make dystrophin in order to maximize the overall affect of improving muscle function in individuals with Duchenne's.

Below are a few of the different stem cell types that scientists are currently experimenting with to assess whether they meet the ideal criteria for treating this disease.

Skeletal muscle precursors

Myoblasts

In 1991, clinical trials provided proof-of-principle that transplanted stem cells could produce dystrophin in boys with Duchenne's. In these very small trials, intramuscular injections of muscle precursor cells, called myoblasts, were able to stabilize or increase the strength of the muscles in boys with the disease. Since then, other studies have shown mixed results, and even when the immune system is suppressed to prevent rejection, there have not been the improvements in muscle strength that were hoped for. In addition, having to deliver intramuscular injections to skeletal muscle all over the body is not ideal, and such injections cannot be used to transplant cells into important respiratory muscles such as the diaphragm.

Satellite cells

Scientists now know that satellite cells are the precursor population responsible for virtually all of the growth, maintenance and repair of skeletal muscles. In response to injury, satellite cells either differentiate into myoblasts that fuse together to build or repair muscle fibres, or self-renew and remain dormant in muscle tissue waiting to be called on to regenerate muscle at a later time.

Early research in mice lacking dystrophin was promising: normal satellite cells injected into the animal's muscle efficiently fuse with the muscle fibres, make dystrophin and also contribute to the satellite cell pool. One ongoing issue is that only small numbers of satellite cells can be extracted from muscle biopsies and it is not easy to expand them in culture. However, recent studies have shown that fetal-like satellite cells generated from embryonic stem cells can be delivered through the vascular system and effectively contribute to the repair of regenerating muscles in mice (see Pluripotential stem cells below). Satellite cells represent the best candidate for stem cell therapy but difficult challenges exist in utilizing the cells for therapy.

Mesangioblasts

Mesangioblasts come from a population of stem cells called pericytes that are associated with the blood vessels in skeletal muscle. Studies in mice and dogs have corroborated that mesangioblasts can be delivered systemically by injection into the blood, can partially restore dystrophin levels in the target muscles, and possibly even buttress the stem cell pool of satellite cells. Results from a phase I clinical trial testing the safety and efficacy of muscle pericyte preparations are soon to be published.

Bone marrow stem cells

Bone marrow stem cells can also differentiate into muscle-forming cells and studies in mice have shown that these cells can find areas of muscle degeneration and contribute to muscle repair. Two of the main advantages of bone marrow stem cells over skeletal muscles stem cells are that they are more easily harvested and systemic delivery is already routinely possible. However, the levels of contribution are extremely low making this approach not attractive at this time.

Recently, scientists have co-opted bone marrow stem cells to deliver a gene therapy called 'MAGIC-Factor-1' which was shown to repair damaged muscle in mice. Phase I clinical trials using bone marrow stem cells to deliver MAGIC-

Factor-1 have so far shown no adverse events. Other trials using bone stem cells to deliver gene therapies are ongoing.

Mesenchymal stem cells

Human mesenchymal stem cells can be harvested from human fat and dental pulp and are amenable to transplantation by intravenous injection into mice and dogs. However, current clinical trials are showing that the extent of their contribution to skeletal muscle regeneration may be limited. In addition, human mesenchymal stem cell grafts tend to elicit an immune response in human recipients. The prospect of lifelong immunosuppression for recipients is not ideal and so researchers are exploring ways around this, such as genetically modifying a DMD patient's own mesenchymal stem cells so that the graft can be autologous.

Pluripotent stem cells

Embryonic stem cells and induced pluripotent stem cells are both capable of generating the myriad different cell types within the body. Although these powerfully regenerative cells are capable of making the necessary cells to repair injured muscle, concerns about the ethics of their use and/or the potential of tumour formation has made their application as therapies proceed with caution. Nevertheless, preclinical studies are encouraging and have shown that it is possible to stimulate embryonic stem cells to make skeletal muscle precursors in a dish and the cells go on to survive and contribute to muscle formation without forming tumours when transplanted into mice. In 2010, researchers collaborating in Japan and USA were able to correct the dystrophin gene defect in skin cells from a patient with DMD and turn the cells into induced pluripotent stem cells. If patient-specific, corrected iPS cells could be stimulated to become muscle stem cells in a dish, they might someday be used as autologous (patient-specific) transplants that could restore dystrophin levels in patients with Duchenne's.

Stem cell scaffolds

In the search for the ideal stem cell to treat Duchenne's muscular dystrophy, researchers have realized that muscle stem cells do a better job of rebuilding muscle when they are embedded in a matrix that resembles the architectural support the cells have in the body. When satellite cells are embedded in biodegradable 3D protein scaffolds, they are able to form normal skeletal muscle in a dish. The ability to expand muscle stem cells and their progenitors in a dish will be an extremely valuable tool for future regenerative therapies.

Canadian contributions

Canadian scientists are advancing basic research on muscle-forming progenitor cells, which they hope will provide new ways for treating Duchenne's muscular dystrophy.

Satellite cells were discovered 50 years ago and since then they have been proven crucial for long-term muscle regeneration. In a comprehensive review paper published in 2012, a team of researchers in Ottawa have taken stock of what is known today about the molecular regulation behind building muscle. The picture that is unfolding is far from black and white. Instead, there appears to be a complex regulatory network driving muscle development in the embryo and facilitating muscle repair in adults. In addition to the regulatory network, the importance of the specialized environment, or niche, that maintains the delicate balance between self-renewal versus differentiation of satellite cells cannot be understated. In fact, the niche is so critical for satellite cells that yanking them away from it might explain why satellite cell transplants have not done so well.

At the Sprott Centre for Stem Cell Research in Ottawa researchers are uncovering some of the important players in the satellite cell regulatory network. Their research on transcription factors that push satellite cells to commit to the myogenic lineage has led them to identify two in particular, PAX3 and PAX7, which they think may be essential regulators for forming muscle and maintaining muscle precursor cells. More and more, the proteins and genes involved in the regulatory network are being studied on a large scale and researchers are using the outcome to better understand the cues that coax satellite cells down the muscle-forming pathway. The knowledge gleaned from these and other

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basic research studies will be key for devising ways to manipulate satellite cells for the purpose of future regenerative therapies.

Clinical studies

Since 1992, there have been a handful of small clinical trials testing the safety and efficacy of stem cells for treating boys with Duchenne's. The majority has focussed on myoblasts or myogenic precursors in the bone marrow. The past five years has not seen many trials occurring. Instead, researchers are busy working out the variables such as stem cell selection, ways to improve systemic delivery, the signals stimulating the formation of muscle progenitors, how best to promote their integration into the muscle niche, and gene transfer techniques. This knowledge will allow them to make the best and safest choices for the future use of stem cells in clinical trials for Duchenne's muscular dystrophy.

Web Resources

- Muscular Dystrophy Association: http://www.mdausa.org/
- Parent MD: http://parentmd.org/
- Muscular Dystrophy Canada: http://www.muscle.ca/
- Your Genes, Your Health: http://www.ygyh.org/dmd/whatisit.htm
- EuroStemCell: http://www.eurostemcell.org/factsheet/muscular-dystrophy-how-could-stem-cells-help
- Muscular dystrophy glossary: http://www.ygyh.org/dmd/glossary.htm
- Jesse's Journey: http://www.jessesjourney.com/

Selected Reading List

- Regenerative Medicine. Department of Health and Human Services. August 2006, chapter 4: "Use of Genetically Modified Stem Cells in Experimental Gene Therapy." <stemcells.nih.gov/staticresources/info/scireport/PDFs/F.%20Chapter%204.pdf>
- Pittsburgh scientists identify human source of stem cells with potential to repair muscle: www.eurekalert.org/pub_releases/2007-09/chop-psi090407.php
- Parent Project Muscular Dystrophy: http://www.parentprojectmd.org/site/PageServer?pagename=nws_index
- BIO-NMD newsletter, issue 3 (March 2012) [.PDF]: http://www.bio-nmd.eu/userfiles/BIONMD03.2.pdf