RESEARCH update

Muscular Dystrophy Canada

Spring 2007 Compiled by Bill Tillier.

Research updates reflect a sampling of what is important and new in muscle research.

Recent Developments

► Skeletal muscle displays tremendous resiliency and regenerative capabilities allowing it to recover from a number of injuries. There appears to be a complex series of proteins that stimulate regeneration and another series of proteins that inhibit muscle regeneration. In this way, a balance is achieved, maintaining the proper amount of muscle and avoiding the overproduction of muscle. In the case of muscle disease, it appears that this regeneration is somehow inhibited. Recent research demonstrates that increases in a chemical (cytokine TGF-β) within the muscle inhibits this regeneration and causes fibrosis in a number of muscle diseases. Fibrosis involves development of excess fibrous connective tissue in the muscle. In mouse models of Marfan syndrome and Duchenne muscular dystrophy, this chemical can be blocked by a drug called losartan, thus restoring muscle regeneration, reducing fibrosis and improving muscle function. This research suggests that losartan could potentially be used to slow the progression of muscle weakness in Duchenne and in other muscle conditions. Losartan is a well-known medication, used to treat hypertension. Because the drug is well known, clinical trials in humans could proceed quickly to determine if this medication will help muscular dystrophy patients or not.

Reference: Cohn, R.D. et al. Angiotensin II type 1 receptor blockade attenuates TGF-b–induced failure of muscle regeneration in multiple myopathic states *Nat. Med.* 13, 204–210 (2007).

► SMA: Research often uses mice that have been bred or genetically manipulated to simulate human diseases. This research uses a mouse model of the motor neuron disease spinal muscular atrophy (SMA). SMA is a common disorder affecting one out of every eight to ten thousand children and it is also a severe disorder, in the most common form,

called type 1, children usually pass away by the age of two. Scientists know that SMA is caused by mutations in genes that result in reduced protein production, impairing muscle function. This research is examining medications designed to increase the amount of protein produced by these genes, specifically a drug called trichostatin A (TSA) that is in a class of drugs called histone deacetylase (HDAC) inhibitors. Mice treated with this medication were larger and had better function than mice that did not receive treatment. The article ends with this quotation: “we demonstrated that the potent and selective HDAC inhibitor TSA significantly improved motor performance, attenuated weight loss, increased survival, and improved the pathology of the motor unit in SMA model mice. Our results provide a strong preclinical basis for examining the newer, more potent HDAC inhibitors in clinical trials in SMA patients.”

Reference: Avila AA, Burnett BG, Taye AA, Gabanella F, Knight MA, Hartenstein P, Cizman Z, Di Prospero NA, Pellizzoni L, Fischbeck KH, Sumner CJ. "Trichostatin A increases SMN expression and survival in a mouse model of spinal muscular atrophy." *The Journal of Clinical Investigation*, Advance Online Publication, February 22, 2007, doi: 10.1172/JCI29562 (will appear in the March issue).

► Charcot-Marie-Tooth disease (CMT): CMT affects 1 in 2,500 individuals causing nerves and muscles in the feet and hands to weaken resulting in numbness and, in many cases, loss of function. CMT affects the peripheral nerves, among the longest nerves in the body and where energy transport is especially important. In the cell, normally it is the job of mitochondria to produce energy. It was assumed that some sort of defect in the mitochondria was leading to a reduction of energy production, causing CMT. New research shows that mutations in a gene (Mitofusin 2 (MFN2)), known to cause one form of CMT, also disrupts the movement of the cell's mitochondria. A minor disruption in mitochondria movement reduces the energy supply, weakening or even killing the cell. This gives researchers important clues were to search for causes and possible treatments (in mitochondria movement disruption and not in some internal defect in mitochondrial energy production).

Reference: Robert H. Baloh, Robert E. Schmidt, Alan Pestronk, and Jeffrey Milbrandt Altered Axonal Mitochondrial Transport in the Pathogenesis of Charcot-Marie-Tooth Disease from Mitofusin 2 Mutations. *J. Neurosci.*, Jan 2007; 27: 422 - 430 ; doi:10.1523/JNEUROSCI.4798-06.2007.

► This research on inclusion body myositis (IBM) is from the laboratory of Dr. Giulio Cossu in Italy. It is important for IBM but also because the general approach used here may also be applied to Duchenne muscular dystrophy (see following article as well).
Point Summary
- The cause of IBM has not been discovered although much is already known about the changes that take place in the IBM muscle.
- IBM has two major aspects, one having to do with the immune system attacking and killing muscle cells, the other, a deterioration of the muscle cells' proteins.
- Efforts to use drugs to help reduce the immune system reaction have failed to improve IBM patients.

- Mesoangioblasts, vessel-associated stem cells, are able to differentiate into a variety of tissues including skeletal, cardiac and smooth muscle. Previous research has shown that when delivered into an artery, mesoangioblasts restore, to a significant extent, muscle structure and function in a mouse model of muscular dystrophy.
- This study describes the isolation and functional characterization of mesoangioblast cells obtained from the muscle biopsies of IM patients and shows that in IBM, mesoangioblasts fail to develop into skeletal muscle (myotubes).
- The growth and repair of muscle is normally controlled by a complicated series of steps involving a master gene called MyoD.
- A number of smaller genes control MyoD function and turn it on and off as required by the muscle, for example, as muscle is damaged, MyoD is turned on to replenish it.
- These researchers have discovered that MyoD is not functioning in the skeletal muscle (in the arms and legs) of IBM patients, thus muscle replenishment can not take place.
- Researchers discovered a small gene (BHLHB3) that turns off MyoD is overactive in IBM muscle and that once it becomes overactive it keeps turning itself on, perpetuating the inhibition of the MyoD and thus blocking mesoangioblasts from developing into skeletal muscle.
- The problem of reduced MyoD function in IBM can be addressed in two ways:
- First, through cell transplantation. The researchers obtained human muscle biopsy-derived IM mesoangioblasts (from DM and IBM) and treated them in the laboratory with a virus (an adenoviral vector) carrying with it the full-length mouse version of MyoD. In other words, the researchers took the genetic code for MyoD from mice and used the virus to put it into these IM mesoangioblast cells. Three types of cells were then transplanted into the muscles of special research mice, two using DM mesoangioblasts, two using untreated (wild) IBM mesoangioblasts and two using the treated (adenoMyoD-transduced) IBM mesoangioblasts. The IBM mesoangioblasts treated with the adeno-MyoD resulted in a significant improvement of muscle function when this group was compared with the group of muscles injected with wild-type (untreated) IBM cells. Thus, the introduction of MyoD overcomes the BHLHB3 inhibition. It also activates the MyoD that is already present in the cells, irreversibly making the cells generate new muscle. Ideally, future treatments will use mesoangioblast cells obtained from a patient, treated in the laboratory and injected back into the patient, restoring muscle production and thus avoiding any immune reactions to the injected cells.
- Second, the researchers examined the effect of using a genetic method to block the function of the BHLHB3 gene. The researchers used small interfering RNA (siRNA) created specifically to block the action of BHLHB3 mRNA in mesoangioblasts obtained from three IBM patients that were subsequently grown in the laboratory for seven days. The siRNA treated cells were able to differentiate into new muscle.
-Either knocking out this little inhibitory gene, or adding MyoD, would not "cure" the IBM, but it would partly compensate for IBM's effects by allowing the generation of new muscle fibers and thus improving muscle function.
- Until they can discover the root causes of IBM and address them directly, the goal is to either silence the overactive BHLHB3 gene or use this cell-based therapy to increase MyoD and thereby improve muscle function. In addition, this approach can be combined with future drugs aimed at addressing the faulty immune response that attacks muscle cells in the first place.

- Also, ultimately and ideally, taking mesoangioblasts and manipulating them in the laboratory with different mixtures of growth factors and biomolecules may greatly increase differentiation and increase their therapeutic efficacy, without requiring the added complexity of using genetic manipulation or viral infection.

Reference: Roberta Morosetti et al MyoD expression restores defective myogenic differentiation of human mesoangioblasts from inclusion-body myositis muscle *PNAS* | November 7, 2006 | vol. 103 | no. 45 | 16995-17000

► Duchenne muscular dystrophy: An important article on Duchenne muscular dystrophy was published in November, 2006. Scientists conducted research on golden retriever dogs that display a crippling form of dystrophy very similar to the human form of Duchenne muscular dystrophy. In this research, conducted by Dr. Giulio Cossu and his team in Milan Italy, a special type of stem cells were taken from healthy dogs and injected into diseased animals. These stem cells, called mesoangioblasts, are found in the walls of blood vessels and have previously been used to produce muscle cells. Previous research by the Cossu team has shown that these cells can help restore muscle function in mice, bred to show Duchenne muscular dystrophy. Researchers were therefore anxious to try the technique in these dogs as they more closely resemble the human form of the disease. Researchers extracted mesoangioblasts from normal dogs and expanded them in the laboratory, then injected them into the sick dogs over a five-month period. The injected cells traveled through the blood and into many of the dog's muscles (but some muscles more than others) where they fused with existing fibers. This created additional dystrophin protein thereby rejuvenating the fibers and restoring function to a dramatic degree. More research must be done on dogs, first to discover if these cells are safe and to ensure that there are no side effects, then to try to increase the efficiency of the technique. Injecting the cells directly into the blood is a huge advantage, as previous efforts have attempted to inject cells into the individual muscles and this poses a tremendous challenge. Eventually, researchers want to take these stem cells directly from the patient and use gene therapy to repair the cells and then inject them back into the patient, thus avoiding issues of immune rejection. Mesoangioblasts present a distinct advantage over other types of muscle cells (satellite cells) because they can cross the vessel wall which, together with easy isolation and good muscle generation potential, make them an ideal candidate for muscle rehabilitation therapy using cell transplantation. Another potential use of mesoangioblasts, given their extraordinary capability to diffuse throughout the body with blood and to integrate in muscle, is to use them as vectors to deliver materials into the muscle. The authors conclude by saying, "Thus, the work reported here sets the logical premise for the start of clinical experimentation that may lead to an efficacious therapy for Duchenne muscular dystrophy."

Reference: Sampaolesi, M. et al. Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. *Nature* 444, 574–579 (2006).

► Healthy muscle demonstrates a complicated balance between muscle protein degeneration (through wear and tear or injury) versus muscle protein production (in response to exercise or to repair injury). Many factors are being discovered that both

promote and inhibit this protein production. This study describes two chemicals that can speed up the regeneration of damaged muscle and therefore represent potential targets for medication development to treat people with muscle wasting diseases. The first is called NF-kB. This chemical is well known as for its role in inflammation and it turns out that inflammation plays a critical role in maintaining muscle mass. After injury or inflammation, NF-kB stops the production of proteins and initiates their breakdown, which leads to the loss of muscle mass. By blocking the NF-kB, muscle is protected from wasting and the healing of damaged muscle improves. Protection against muscle wasting was even stronger when a gene producing growth factor IGF-1 was added to muscle tissue with blocked NF-kB. Thus, blocking NF-kB along with administering growth factors like IGF-1, appears to be a promising combination therapy against muscle diseases. Although this research was conducted in mice, the chemicals are very similar in humans.

Reference: Foteini Mourkioti, Paschalis Kratsios, Tom Luedde, Yao-Hua Song, Patrick Delafontaine, Raffaella Adami, Valeria Parente, Roberto Bottinelli, Manolis Pasparakis, and Nadia Rosenthal Targeted ablation of IKK2 improves skeletal muscle strength, maintains mass, and promotes regeneration *J. Clin. Invest*. 2006 116: 2945-2954.

► X-linked spinal and bulbar muscular atrophy (SBMA or Kennedy disease) is an inherited neurodegenerative disorder. This research reports results suggesting a better therapeutic and preventative approach to treating SBMA, by using a drug (ASC-J9) to disrupt the pathway of the disorder.

Reference: Zhiming Yang ASC-J9 ameliorates spinal and bulbar muscular atrophy phenotype via degradation of androgen receptor *NATURE MEDICINE*, 13, 2007, 348-353.

►PTC Therapeutics, Inc. (PTC), a biopharmaceutical company announced it has been awarded a two-year grant to support costs related to the Phase 2 clinical trial of PTC124 for the treatment of Duchenne muscular dystrophy (DMD). The same company is already in clinical trials of PTC124 for the treatment of cystic fibrosis. PTC124 is an orally administered product designed for the treatment of genetic disorders resulting from to nonsense mutations, mutations consisting of single-point alterations in the genetic code that prematurely stop the process of translation, thereby creating a partial, non-functional protein. This approach is called GEMS (Gene Expression Modulation by Small-molecules). The Duchenne trial began in January 2006. Preliminary results are positive and suggest increases in dystrophin in muscle biopsies in a number of patients. The preliminary data were presented at the PPUK 4th International DMD Conference in London, England, October 21, 2006. “These results are the first example of an oral therapy addressing the underlying cause of DMD by restoring dystrophin production,” said Dr. Richard Finkel, Director of the Neuromuscular Program, Children’s Hospital of Philadelphia, PA, one of the trial’s lead investigators. “These preliminary results are very encouraging and add to the growing body of clinical evidence supporting the potential of PTC124 as a treatment for genetic disorders due to a nonsense mutation,” said Stuart W. Peltz, Ph.D., President and Chief Executive Officer of PTC Therapeutics. “The findings

in the DMD trials are consistent with the results observed in Phase 2 clinical trials of PTC124 in patients with cystic fibrosis. We intend to extend this concept into other nonsense-mediated genetic disorders."

Reference: http://www.ptcbio.com/default.aspx
http://www.medicalnewstoday.com/medicalnews.php?newsid=54639

► Inside cells, chemicals are often assembled on scaffolds made out of small proteins called caveolins. Caveolin-3 is a form of scaffold protein found in muscle. Mutations in the gene that produces caveolin-3 cause autosomal dominant limb-girdle muscular dystrophy 1C (LGMD1C), however, the mechanism of this disease was not understood. Dr. Yoshihide Sunada and his team from Kawasaki Medical School, Japan, show that loss of caveolin-3 in a mouse model of muscular dystrophy increases the activity of myostatin inside the cell. Myostatin is a well known inhibitor of muscle growth that ultimately leads to muscle wasting. The study shows that the over-activation of myostatin plays an important role in causing muscular dystrophy in mice, and that a major function of caveolin-3 in skeletal muscle is to prevent myostatin function, thereby preventing muscle wasting. The researchers also show that the inhibition of myostatin reverses the muscle wasting in these mice implying that inhibiting myostatin in patients with LGMD1C may be a treatment possibility.

Reference: Yutaka Ohsawa, Hiroki Hagiwara, Masashi Nakatani, Akihiro Yasue, Keiji Moriyama, Tatsufumi Murakami, Kunihiro Tsuchida, Sumihare Noji, and Yoshihide Sunada Muscular atrophy of caveolin-3–deficient mice is rescued by myostatin inhibition *J. Clin. Invest.*, Nov 2006; 116: 2924 - 2934.

► Attempts to treat muscle disorders increasingly are focused upon trying to boost muscle function to counteract the effects of the disease without necessarily slowing down or stopping the actual disease process. Until specific causes and treatments for these diseases can be developed, this approach may significantly help improve practical muscle function in people with muscular disorders. An ongoing collaboration of scientists has recently published the results of research using a drug to counteract the effects of muscular dystrophy in mice. Scientists used two mouse models of muscular dystrophy, one that naturally develops a disease similar to Duchenne muscular dystrophy in humans, the other, a strain genetically altered to develop a form similar to the human limb-girdle muscular dystrophy. The scientists tested a chemical called trichostatin A (TSA), which inhibits an enzyme called deacetylase. TSA is currently under clinical study for treating breast cancer. Mice with MD given TSA daily for two to three months were virtually indistinguishable from healthy mice, and research showed virtually no difference between the muscle strength of the mice with MD given the drug versus the healthy mice. Several advances led to this recent research -- for example, previous research had shown that a chemical called follistatin had a role in muscle development – the follistatin blocks the activity of another protein called myostatin, leading to increased muscle production. When researchers treated muscle cells with deacetylase inhibitors (TSA), they saw that follistatin was over-produced resulting in larger cells. Therefore, by increasing follistatin, researchers can inhibit myostatin leading to muscle regeneration. More research is needed

to determine how long the drug works and if it works in larger animals with bigger muscles, such as dogs, before such drugs can be tested in people.

Reference: Minetti GC, et al. Functional and morphological recovery of dystrophic muscles in mice treated with deacetylase inhibitors. *Nat Med* 2006 Sept 17; PMID 16980968.

► Myotonic muscular dystrophy is caused by a mutation in DNA that results in a number of CTG sequences being repeated in a particular gene (DMPK). Normally, the DNA code creates RNA that then leaves the cell nucleus and goes on to direct the assembly of protein inside the fluid of the cell (the cytoplasm). Current thinking is that in myotonic dystrophy, the abnormal DNA produces abnormal RNA that stays within the nucleus of the cell and interferes with the proper transport of other proteins in and out of the cell's center. This research, conducted by Dr. Mani Mahadevan, demonstrates that the RNA produced by the mutated DNA appears to be toxic to muscles. Researchers developed a mouse model designed to create abnormal RNA. Researchers demonstrated that poisonous RNA lead to the various effects of myotonic dystrophy and that the removal of this RNA allowed the mice to return to normal. Although the mutation would be found in every cell, apparently the toxic RNA is not found uniformly, it appears at higher levels in muscle cells, in the heart and brain, in the lining of the intestines and in the lens and muscles of the eyes. Dr. Mahadevan believes that a treatment to reduce the expression of this toxic RNA molecule may be a viable treatment for myotonic dystrophy.

Reference: Mani S Mahadevan, Ramesh S Yadava, Qing Yu, Sadguna Balijepalli, Carla D Frenzel-McCardell, T David Bourne & Lawrence H Phillips Reversible model of RNA toxicity and cardiac conduction defects in myotonic dystrophy *Nature Genetics* September 2006, Volume 38 No 9 pp1066 – 1070

► Book review: Duchenne Muscular Dystrophy: Advances in Therapeutics edited by J.S. Chamberlain and T.A. Rando, 450 pp., Taylor and Francis Group, 2006

“This timely book has an introduction by A.E.H Emery and is then divided into four sections with individual chapters being written by one or more experts in that field. One of the major strengths of the book is its simple, logical layout. Section I (“Duchenne Muscular Dystrophy Background”) encompasses a clinical overview of DMD, the role of dystrophin and the dystrophin-associated proteins. Section II deals with diagnostic considerations, including protein studies, while Section III moves on to current therapeutics, including rehabilitation management. Section IV (Experimental Therapeutics) provides a comprehensive summary of current therapies under investigation. This section starts with a particularly useful chapter by Dominic J. Wells entitled “Therapeutic Principles and Challenges in Duchenne Muscular Dystrophy” which gives an overview of the topics, such as pharmacological approaches and use of viral vectors, to follow. It also contains a short but excellent summary of the animal models available and their limitations. The structure makes it easy to locate areas of interest and, as each chapter deals with a separate topic, chapters can be read as “stand-

alone" pieces. This does, however, lead to some repetition of themes, particularly in the introductory paragraphs of certain chapters.

For research clinicians, the section on current therapeutics was useful, but many of the highlights were in the section on experimental approaches. The chapters on oligonucleotide-mediated exon skipping and gene editing (Carmen Bertoni and Thomas A. Rando) and adenoviral-mediated gene therapy (Laura Goldberg and Paula R. Clemens) were very clear and benefited from good diagrams. Chapter 14 on cellular mediated delivery (K. Liadaki, F. Montaro and L.M. Kunkel) was notable for turning the complex area of myogenic progenitor cell-marker expression into an understandable and readable chapter.

This book will naturally appeal to research clinicians but its breadth of scope means that there is much to recommend it to laboratory scientists and to clinicians caring for boys with DMD. The highly technical nature of some chapters does, however, introduce a lot of terminology with which some potential readers may not be familiar, possibly reducing the impact of some sections.

In summary, for anyone interested in the progress of research into DMD, this book is a wonderful summary of the current literature and is extremely well-referenced. Its portability (A5 format) and structure will encourage readers to "dip in" as and when they wish."

Reference: Duchenne Muscular Dystrophy: Advances in Therapeutics Review by: Garrood, Penny MD; Bushby, Kate MD, *Neurology*. 67(3):546, August 8, 2006.

► One of the tantalizing possibilities of stem cell research is that scientists will learn to control stem cell differentiation allowing them to transform one type of cell into another. Researchers recently transformed adult human adipose – fat tissue – stem cells into smooth muscle cells. Smooth muscle is important in the function of many organs including the intestine, bladder and blood vessels. The study shows that adipose stem cells can be transformed to express the physical, biochemical and functional characteristics of smooth muscle cells. The study supports the idea of using fat stem cells for smooth muscle tissue engineering and repair.

Reference: Larissa V. Rodríguez, Zeni Alfonso, Rong Zhang, Joanne Leung, Benjamin Wu, and Louis J. Ignarro Clonogenic multipotent stem cells in human adipose tissue differentiate into functional smooth muscle cells *PNAS* 2006 103: 12167-12172; published online before print as 10.1073/pnas.0604850103.

► A key characteristic of myotonic dystrophy is that the muscle is hyper-excitable – once the muscle contracts it is difficult to release. Research has shown that mutated genes block the action of critical proteins within the cells. One such critical protein is called muscleblind, a protein needed to help muscle and eye cells mature. This protein sticks to deformed copies of RNA molecules that build up within the cells center nucleus. Researchers discovered that newborn muscle is quite different than adult muscle. After

birth, muscle proteins undergo a transition leading to the adult configuration. This transition appears to be blocked in myotonic dystrophy. Adult myotonic patients display fetal muscle forms of protein. Muscleblind proteins are involved in the transition from newborn to adult proteins. When researchers injected extra copies of muscleblind protein into the muscle of genetically altered mice, they were able to correct the myotonia. Future research will try to introduce muscleblind protein to all muscles in the body through the bloodstream. It is hoped that increasing levels of muscleblind protein might prevent the muscle deterioration characterizing adult myotonic illness. The researchers caution that transferring this research to humans is a distant goal.

Reference: Rahul N. Kanadia, Jihae Shin, Yuan Yuan, Stuart G. Beattie, Thurman M. Wheeler, Charles A. Thornton, and Maurice S. Swanson Reversal of RNA missplicing and myotonia after muscleblind overexpression in a mouse poly(CUG) model for myotonic dystrophy *PNAS* 2006 103: 11748-11753; published online before print as 10.1073/pnas.0604970103

► Researchers have recently used a common drug, normally used to treat epilepsy, called valproate, to treat adult patients with spinal muscular atrophy. Previous research had shown that the drug increases levels of a key protein in the laboratory (SMN protein) by increasing the function of SMN2 genes. Researchers were therefore encouraged to test valproate in the types of SMA related to the SMN2 gene. These are usually types that develop later in life, hence the focus on adult patients. More research will be required to ascertain the effectiveness of this approach.

Reference: Conrad C. Weihl, Anne M. Connolly, and Alan Pestronk Valproate may improve strength and function in patients with type III/IV spinal muscle atrophy *Neurology* 2006 67: 500-501.

► Theories of FSHD proposed in 1992 suggested that the mutations seen in chromosome 4q cause increased activity of one or more nearby critical genes, resulting in a series of events eventually producing the disease. Recent research, combined with a number of previous studies, now demonstrates there is substantial evidence against the overexpression of any known gene in the region of the FSHD mutation. Further research will be required before a better understanding of the mechanism of FSHD can be achieved.

Reference: see the editorial by Steven A. Greenberg, MD; and George W. Padberg, MD, PhD. Pushing the genetic frontier with facioscapulohumeral muscular dystrophy. *Neurology* 2007; 68:544–545.

► Institute of Musculoskeletal Health and Arthritis (IMHA). IMHA supports research to enhance active living, mobility and movement, and oral health; and addresses causes, prevention, screening, diagnosis, treatment, support systems, and palliation for a wide range of conditions related to bones, joints, muscles, connective tissue, skin and teeth. IMHA is part of the Canadian Institutes of Health Research (CIHR), the principal source of government of Canada health care funding.

Reference: http://www.cihr-irsc.gc.ca/e/13217.html

► Researchers have recently discovered a new gene that mutates to cause nemaline myopathy. The gene produces the protein cofilin 2. Like the five other genes previously associated with this disease, this gene produces a protein (cofilin 2) that is found inside the muscle fiber, in an area where muscle filaments slide over each other to produce muscle contractions.

Reference: Pankaj B. Agrawal, et al. Nemaline Myopathy with Minicores Caused by Mutation of the CFL2 Gene Encoding the Skeletal Muscle Actin–Binding Protein, Cofilin-2. *Am. J. Hum. Genet.*, 80:162-167, 2007

Review articles

Periodically, an expert will write a comprehensive review, updating what is currently known about a given topic, usually summarizing the causes of a given disease, including any advances in treatment.

► In this review, researchers describe the molecular tactics aimed at the specific targeting of the mutant mRNA in dominant diseases. There are numerous examples in the literature of gene therapy applications for recessive disorders. There are few instances, however, of studies conducted to treat dominantly inherited pathologies. This is because there are fewer cases of dominantly inherited diseases and because it is far easier to correct recessive mutations than dominant ones. Typically recessive mutations cause a loss of (or reduced) gene function which can be compensated for by introduction of a replacement [gene] allele into the cell. In contrast, dominant negative mutations not only display impaired function, but also exhibit a novel one that is pathologic to the cell. Treating these conditions by gene therapy implies having to inhibit the dominant allele without altering the expression of the [normal] wild-type gene. This review describes different strategies aimed at silencing dominant mutations through mRNA destruction and provides examples of their application to known autosomal dominant diseases. An overview of the most common molecular approaches is presented.

Reference: Richard Pelletier, Solenne O.P. Caron and Jack Puymirat RNA Based Gene Therapy for Dominantly Inherited Diseases *Current Gene Therapy*, 2006, 6, 131-146 131 1566-5232/06

► Researchers performed a systematic review to identify studies that reported the accuracy of tests for the diagnosis of myasthenia gravis.

Reference: Michael Benatar A systematic review of diagnostic studies in myasthenia gravis *Neuromuscular Disorders* 16 (2006) 459–467.

► One of the main features of the muscle cell is the dystrophin–glycoprotein complex (DGC). This complex is found in the wall of the muscle cell (the sarcolemma) and is built from many many proteins that interact with each other, almost like the gears in a clock.

The complex forms the critical link between the structure within a cell (cytoskeleton) and the area just outside of the cell (extracellular matrix). These complexes are characteristic of muscle cells but are also found in other types of cells as well. Many of the major muscular dystrophies have been linked to defects in one of these DGC associated proteins. For example, dystrophin protein defects lead to Duchenne muscular dystrophy and Becker muscular dystrophy. Defects in other proteins are commonly associated with limb girdle muscular dystrophies. The precise function of the DGC is still debated and it is quite possible that more DGC proteins await discovery.

Reference: Clare L. Batchelor and Steve J. Winder Sparks, signals and shock absorbers: how dystrophin loss causes muscular dystrophy *TRENDS in Cell Biology* Vol.16 No.4 April 2006

► Gene therapy requires some sort of delivery system – a “bare” gene can not simply be introduced into the body. Commonly, viruses are used as a delivery system; these delivery systems are called vectors. The piece of gene is attached to the viral gene code and, as the virus enters the body, it carries the gene with it into the cells. In addition to a vector, an appropriate target needs to be developed. Skeletal muscle is an important target for gene delivery as this approach may be used for the treatment of muscular disease or to induce proteins into the vascular system. This review focuses on the major developments in viral and non-viral vectors over the last couple of years and in particular, on the advances in vascular delivery to multiple muscles from a single injection. Finally, examples of two diseases (Duchenne muscular dystrophy and hemophilia) for which muscle-directed gene transfer has entered clinical trials are provided.

Reference: Dominic J Wells Viral and non-viral methods for gene transfer into skeletal muscle *Current Opinion in Drug Discovery & Development* 2006 9(2):163-168.

► Human muscle stem cells: Stem cells are unspecialized cells that have been defined in many different ways but they have two important characteristics that distinguish them from other cells in the body. First, they can replenish their numbers for long periods through cell division. Second, after receiving certain chemical signals, they can produce, daughter cells that can differentiate or transform into specialized cells with specific functions, such as heart, nerve or muscle. In recent years, stem cells have received much attention owing to their potential use in cell-based therapies for human neurodegenerative diseases such as Parkinson’s disease, stroke and muscular dystrophies. However, many questions need to be resolved before stem cells with myogenic potential are used in clinical standard protocols.

Reference: Racquel N Cooper, Gillian S Butler-Browne and Vincent Mouly Human muscle stem cells *Current Opinion in Pharmacology* 2006, 6:295–300

► Several muscular disorders commonly display cognitive and psychiatric deficits, in particular, Duchenne muscular dystrophy and myotonic dystrophy. This article indicates that estimates for the prevalence of mental retardation in the normally developing population are approximately 9%, whereas in children with DMD estimates have ranged

from 20% to as high as 50% in some studies. Myotonic dystrophy type 1 may involve skeletal muscles, lens, heart, lungs, gastrointestinal tract, bone, skin, and the central and peripheral nervous system. The central nervous system symptoms of DM1 may include cognitive impairment, hyper-somnolence (increased sleep), heightened sensitivity to anesthetic agents, neuroendocrine dysfunction, and personality and behavior disturbances. Some of these symptoms, such as mental retardation in individuals with congenital DM1, occur during development; others, such as hyper-somnolence, appear during adult life. The article reviews cognitive impairment in neuromuscular disorders.

Reference: Maria Grazia D'angelo, and Nereo Bresolin. Cognitive Impairment in Neuromuscular Disorders. *Muscle Nerve* 34: 16–33, 2006.

► Here is the latest review article on FSHD: RABI TAWIL, and SILVERE M. VAN DER MAAREL, Facioscapulohumeral Muscular Dystrophy *Muscle Nerve* 34: 1–15, 2006.

Also: an excellent layperson summary of FSHD entitled "Impossible Things" can be found in the March - April 2007 issue of Quest Magazine from the MDA (USA), pages 28 – 34, available for viewing or download on their website at: http://www.mdaquest-digital.com/mdaquest/20070304/?pg=28

► Laminopathies: The center of the cell, called the nucleus, is surrounded by a wall called the nuclear lamina. This wall is made out of proteins. The wall regulates the movement of molecules into and out of the nucleus (where the DNA of the cell is housed), and researchers believe it may play a role in regulating the activity of certain genes. The lamina proteins are produced by a set of genes and over 180 mutations in these genes are associated with at least 13 known diseases, called the laminopathies, which include several forms of muscular dystrophy (types of Emery–Dreifuss muscular dystrophy, Limb girdle muscular dystrophy, and Charcot–Marie–Tooth disease). These review articles discuss what is known about these mutations and the resulting disorders.

Reference: Brian C. Capell and Francis S. Collins. Human laminopathies: nuclei gone genetically awry *Nature Reviews Genetics* December 2006, volume 7, pages 940 – 952.

Also: Brian Burke1 and Colin L. Stewart The Laminopathies: The Functional Architecture of the Nucleus and Its Contribution to Disease *Annu. Rev. Genomics Hum. Genet.* 2006. 7:369–405.

► Cells interact with each other by sending messages back and forth to each other. Inside the wall of the cell is a series of channels (ion channels) formed from donut shaped proteins that carry chemical signals in and out of the cell. These chemicals, called ions, have electrical charges that control the flow in and out of the channels. Channels come in several types, based on the ions that pass through them: positively charged sodium, calcium, or potassium ions and negatively charged chloride ions. These ion channels play a critical role in coupling excitation at the neuromuscular junction to activation of contractile elements within a muscle fiber. Dysfunction of the calcium, chloride,

potassium, or sodium channels of nerve or muscle cells are associated with several disorders (the channelopathies). Abnormal channel function can lead to either muscle paralysis or delayed muscle relaxation (myotonia). For example, the cause of the genetic disorder myotonia congenital, is believed to be an abnormality in the chloride channels of muscle cells (chloride ions are required for a muscle to relax). The abnormal chloride channels also cause an accumulation of potassium outside the cells and an activation of sodium channels in the muscle cells (sodium ions trigger muscle contraction). When the cells have too much sodium but not enough chloride, abnormal repetitive electrical discharges cause stiffness called myotonia. Here is the latest review article on channelopathies: Stephen C. Cannon, Pathomechanisms in Channelopathies of Skeletal Muscle and Brain. *Annu. Rev. Neurosci.* 2006. 29:387–415.

► Here is the latest review article on RNA-Mediated Neuromuscular Disorders (Myotonic Muscular Dystrophy): Laura P.W. Ranum1 and Thomas A. Cooper. RNA-Mediated Neuromuscular Disorders *Annu. Rev. Neurosci.* 2006. 29:259–77.

► Here is the latest review article on Sporadic Inclusion Body Myositis: Marinos C Dalakas. Sporadic Inclusion Body Myositis-Diagnosis, Pathogenesis and Therapeutic Strategies. *Nat Clin Pract Neurol.* 2006;2(8):437-447.

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