Preserving and Building Muscle Fibers

Strategies to fight muscle-fiber fragility have the potential to treat many forms of muscular dystrophy

by Margaret Wahl

We hear a lot these days about genes being destiny, about what can and can't be done to go beyond predetermined biological limits.

A child born with a mutation in the gene for the muscle protein dystrophin, for instance, is destined to develop **Duchenne** or **Becker muscular dystrophy**, depending on the precise mutation and other factors.

Similarly, a child whose genetic heritage leads to a deficiency of the proteins laminin 2 or one of the sarcoglycans is almost certain to develop **congenital** or **limb-girdle muscular dystrophy**, respectively.

But all muscular dystrophies, regardless of their precise genetic origin, have at least one thing in common: fragile or flawed skeletal muscle fibers in which, over time, damage and degeneration outpace repair and regeneration. And this common feature may be an opportunity to change the "destiny" of muscle disease.

Changing fate

There are a variety of strategies being used in the quest to treat or cure muscular dystrophy. One strategy is to correct the basic genetic defect, either by inserting new, functional genes or by repairing existing genetic information. MDA is pursuing these gene therapy strategies avidly.

Other strategies include inserting healthy muscle stem cells that would contribute to the repair and regeneration process in muscle fibers; and stimulating muscle tissue to make more repair cells. Those strategies also are high among MDA's funding priorities.

But there may be another way to change the fate of a muscle fiber that's affected by a muscular dystrophy: redirecting some of the damaging processes that occur as a result of a variety of genetic mutations. Many of those processes are common to multiple forms of muscle disease.

For instance, what if you could put a brake on a normal protein that limits muscle regeneration? What if doing that also could keep an injured muscle fiber from forming so much scar tissue? Or, what if blood flow to contracting muscles could be increased, to improve exercise tolerance and reduce injury?

These and other strategies for protecting and building muscle are described on the following pages, through the eyes of the researchers who have devoted their careers to studying them. Additional strategies can be found online at quest.mda.org.

Note: Although the research highlighted in these articles relied on the readily available and well-described dystrophin-deficient "mdx" mouse (a model of human Duchenne muscular dystrophy), none of the muscle-preserving approaches described is necessarily specific to DMD. Rather, this research has application for many forms of muscular dystrophy and perhaps even other muscle diseases.

As MDA grantee Eric Hoffman puts it, "The primary defect is not the be-all and end-all to everything. It's the initiation of a process, and it's the process — all the things that happen 'downstream' of the genetic defect — that really affects the patient."

Interfering with an Inhibitor of Muscle Growth

Se-Jin Lee, M.D., Ph.D.

Affiliations:
Johns Hopkins University School of Medicine, Baltimore

Strategy:
Inhibiting myostatin to improve muscle growth and strength

Status:
Laboratory experiments

"I was always interested in science, and specifically in science as it applies to medicine," says Se-Jin Lee, a professor in the Department of Molecular Biology and Genetics at Johns Hopkins University School of Medicine in Baltimore.

In 1981, after having graduated with high honors from Harvard with a degree in biochemistry, he went to Johns Hopkins University School of Medicine, earning an M.D. as well as a Ph.D. in molecular biology and genetics in 1989. "Medical school for me was learning about human disease — what we know, what we don't know and what the big problems are that need to be solved," he says.

His next step was an appointment as a staff associate at a private research institute then called the Carnegie Institution of Washington. Now known as the Carnegie Institution for Science, this nonprofit enterprise was created by philanthropist Andrew Carnegie in 1902 as a place where exceptional scientists could be given free rein to pursue their interests.

"They brought people in, gave them some lab space and some money, and basically let them do whatever they felt like for three to five years," says Lee, who started at Carnegie at age 31 and describes the experience as "exhilarating."

The TGF-beta family

Lee soon became interested in the role of secreted proteins in regulating how cells interact with one another, particularly a group of proteins called the TGF-beta family that are known to be potent regulators of cell growth and cell differentiation (maturation).

TGF stands for "transforming growth factor," a protein family that was beginning to receive a lot of attention in the 1980s. In 1989, Lee says, "there were about a dozen members of the TGF-beta family that were known. They had really remarkable biological properties. One subtype could cause bone to form wherever they were implanted. I was interested in doing things that might have some clinical relevance, and the TGF-beta proteins looked really intriguing. I figured there had to be a lot more of them that hadn't yet been identified, so I started to look for new ones."

Lee began the work at Carnegie and then continued it after moving back to Hopkins as a faculty member in 1991.

"We didn't really know anything about these new proteins," he says. "We tried to figure out what they were doing, and one of the approaches we took was to knock out the genes for them in mice and see what happened."

Knocking out number 8

Lee numbered each new suspected TGF-beta family member. One of them, number 8, looked like it might have a role in muscle tissue. "When we knocked out the gene for number 8, the mice developed muscles that were huge," Lee recalls. "Once we realized that growth and differentiation factor (GDF) 8 functioned to limit muscle mass, we decided to rename it 'myostatin.'" (*Myo* is a prefix meaning "muscle," and *statin* means "at rest.")

In 1997, Lee and his colleagues published a paper describing the myostatin discovery and mouse findings in the prestigious journal Nature. Later that same year, he co-authored another paper, showing that cattle bred to have especially large muscles had natural myostatin mutations.

Focusing on myostatin

Lee started focusing more and more effort on myostatin — how it worked and how its activity might be changed to treat muscular dystrophy.

For instance, most secreted proteins land on docking sites called receptors, and Lee wanted to identify the receptor for myostatin. In 2001, he and his colleague Alexandra McPherron at Hopkins published a landmark paper describing how myostatin is maintained in an inactive, latent state, how it interacts with an inhibiting protein called follistatin, and how

Se-Jin Lee

it binds to a receptor on the muscle-fiber surface called activin receptor type IIB, through which it signals the fiber.

In 2002, with Kathryn Wagner (see next page) and others, Lee showed how mdx mice (a model of human Duchenne muscular dystrophy) were stronger and more muscular than expected when they were genetically engineered to lack myostatin.

It was about this time that Lee received a phone call from Markus Schuelke, a neurologist in Berlin who had been called to the delivery room in 1999 to see a newborn baby boy who had unusually large muscles.

"He immediately was struck by the amount of muscle," Lee says. Schuelke remembered having read about myostatin and wondered if he was looking at a child with a myostatin mutation.

"Schuelke was so convinced this was possible that he looked for the mutation," Lee says. "The child's appearance must have been pretty dramatic for him to have gone to all this trouble."

His trouble was well-rewarded. The German baby had two mutated myostatin genes, one from each of his parents, and he was producing almost no myostatin.

When Schuelke asked Lee for help in analyzing the effects of the mutated genes, Lee got involved, and along with many other researchers, Schuelke foremost, published a paper in 2004 that announced to the world that myostatin's role in human muscle was similar to its role in mice and cattle. It also suggested that drastically reducing its level in a child could increase muscle mass without any apparent harm. (According to Schuelke, as of early 2011, the child continues to do well.)

Lee is now a member of MDA's Medical Advisory Committee and has received two MDA research grants since 2004, both focused on understanding how myostatin normally behaves and identifying ways of changing its activity as a potential treatment for muscle diseases. All the myostatin-associated pathways that Lee's group has identified have the potential to become targets for therapeutic development.

"The mouse studies look good," says Lee, who is also encouraged by the health of the myostatin-deficient cattle and the myostatin-deficient child. But, he cautions that humans are not mice or cows and also that there are differences between genetic mutations that exist from birth and later manipulations of proteins or genes.

"I really hope it works," he says of the myostatin-inhibiting strategy now being tested by Acceleron Pharma (see page 9). "But I think there are lots of issues that still have to be resolved. The good news is that the field is moving forward quickly, so we should have at least some answers soon."

Creating a Hospitable Environment for Muscle Regeneration

Kathryn Wagner, M.D., Ph.D.

Affiliations:
Johns Hopkins University, Baltimore
Kennedy Krieger Institute, Baltimore

Strategy:
Reducing fibrosis (scarring) in muscle by inhibiting myostatin

Status:
Laboratory experiments

In May 1997, Kathryn Wagner was doing something she hadn't had much time to do in years: She was watching television. Wagner had just given birth to her first baby, James, and was on leave from her postgraduate training in neurology at Johns Hopkins University School of Medicine in Baltimore.

"I distinctly remember that I was on my bed, with my baby, watching Dan Rather on the evening news show pictures of the 'mighty mouse,'" she recalls, referring to a genetically engineered mouse developed in the laboratory of molecular biologist Se-Jin Lee, who happened to be at Hopkins as well (see page 2).

Mighty mouse led to fruitful collaboration

The so-called mighty mouse lacked a protein called myostatin and had extremely large muscles. "I just thought, 'That has got to be helpful to muscular dystrophy research,'" Wagner recalls.

When she was ready to go back to work, Wagner approached Lee about doing a postdoctoral fellowship in his lab and "we ended up having a fruitful collaboration."

Wagner had earned a doctorate in neuroscience, as well as a medical doctorate, at Hopkins in 1994. During her training in the laboratory of neuroscientist Richard Huganir, she had identified the gene for a muscle protein called dystrobrevin, located in muscle fibers and associated with the dystrophin protein, known to be absent in Duchenne muscular dystrophy.

"Finding something similar to dystrophin and associated with dystrophin made me read the literature and get very excited about muscle and muscle diseases," she says, and her career shifted in that direction.

In 2002, with Se-Jin Lee and others, Wagner, by then an MDA research grantee, published a paper showing that loss of the myostatin protein significantly reduced the severity of the Duchenne dystrophy-like disease that develops in mice that lack dystrophin, known as mdx mice.

Wagner cross-bred myostatin null mice (mice that don't produce myostatin) to mdx mice (mice that don't produce dystrophin), "and I've been working on myostatin inhibition ever since," she says.

Kathryn Wagner

She's now an associate professor of neurology and neuroscience at Hopkins and the director of the Center for Genetic Muscle Disorders at the Hopkins-associated Kennedy Krieger Institute. MDA has given Wagner two additional research grants, both related to reducing myostatin in muscle tissue.

Far less fibrosis when myostatin was gone

Wagner found that mdx mice that lacked myostatin were stronger and more muscular than their mdx counterparts. But there was something else that got her attention: They had far less fibrosis (scar tissue) in their muscles than one would have expected for dystrophin-deficient mice.

Fibrosis is a process in which normal tissue that sustains damage is replaced by fibrous connective tissue. Cells called fibroblasts (generators of fibrous tissue) are the main actors in this process, producing proteins called collagens.

It's a natural phenomenon and may have some value in sealing off parts of an organ to keep an infection from spreading, but, like many biological processes, fibrosis can do more harm than good. Once it gets started, Wagner notes, the original function of the tissue can be destroyed.

Not all muscle diseases involve fibrosis, she says, but it's prominent in DMD and the related Becker muscular dystrophy,

as well as several forms of congenital muscular dystrophy. Fibrosis, Wagner speculates, "may create an environment in which muscle stem cells cannot easily replicate or fuse to form muscle fibers. The proteins that fibroblasts secrete are counterproductive to new muscle formation."

Myostatin, she has found, stimulates fibroblasts, while it suppresses muscle precursor cells called myoblasts. Blocking or removing myostatin does just the opposite: It suppresses fibroblast growth and development, while stimulating myoblasts.

"We actually noticed the antifibrotic effect right from the first experiments in which we cross-bred the mdx mice and the myostatin null mice," Wagner says. "It was clear there was less fibrosis in the mdx animals lacking myostatin. But at that time, we really didn't even suspect that myostatin was acting directly on fibroblasts."

Initially, Wagner says, almost all the excitement about myostatin inhibition focused on the increase in the size of the muscles. But she doesn't think that's as important as other things that myostatin inhibition can do.

"I don't think bigger muscles are necessarily better muscles," she says. "And I think the field is less interested in muscle size now. In the absence of myostatin, there clearly is improved regeneration. That in and of itself is a good thing, and there's this additional benefit of less fibrosis, which is potentially wonderful."

Creating the right environment

Wagner is interested in creating a hospitable environment in which muscle can regenerate, either naturally or with outside help, such as gene therapy, cell transplantation, or other strategies now in development. She believes reducing myostatin levels might even rescue muscle tissue that's already sustained considerable damage and fibrosis.

"In the mdx mouse, we have a lot of data that myostatin inhibitors can reverse fibrosis, even in very old mdx mice," she says. "I don't have any reason to think that wouldn't happen in humans."

Enhancing Blood Flow to Exercising Muscles

Ronald Victor, M.D.

Affiliations:
Cedars-Sinai Medical Center, Los Angeles

Strategy:
Increasing blood flow to exercising muscle by prolonging the action of nitric oxide

Status:
Clinical trial

Ronald Victor admits it: He never set out to study muscular dystrophy. As an adult cardiologist specializing in hypertension (high blood pressure) and neurologic control of cardiovascular mechanisms, he's a relative latecomer to the muscle field, but far from a reluctant one.

Victor, who's now associate director of the Cedars-Sinai Heart Institute and director of the Cedars-Sinai Hypertension Center in Los Angeles, has been interested for decades in how the body allocates blood supply to various tissues under different conditions — something that's largely under the control of the autonomic nervous system.

Ronald Victor

'Fight or flight' vs. 'rest or digest'

The autonomic nervous system, Victor explains, has two divisions, the sympathetic and the parasympathetic. The sympathetic division can be thought of in a general way as helping the body to mount a "fight or flight" response, with an overall increase in heart rate and blood pressure. Pressure increases because blood vessels constrict under sympathetic stimulation.

The parasympathetic division directs the body toward a "rest or digest" mode, generally decreasing heart rate and blood pressure. Under parasympathetic stimulation, blood vessels normally dilate, increasing blood flow but lowering pressure.

In the 1980s, when Victor was training to be a cardiologist at Duke University, physiologists remained somewhat puzzled by the fact that part of the "fight or flight" response involved vascular constriction (vasoconstriction) in some parts of the body, with increased blood flow (apparently vasodilation) in the parts where more blood was needed.

Figuring out how the body manages to constrict blood vessels in some locations and at the same time open them in others was a challenge Victor wanted to meet.

Special blood delivery to working muscles

As he began to research the subject, Victor discovered a 1962 paper describing a phenomenon in laboratory animals called "sympatholysis" — blocking of sympathetic nervous system stimulation — during exercise.

They didn't know what the molecular mechanisms were, but the paper's authors observed that, at least in dogs, sympathetic stimulation resulted in *constricted* blood flow and increased blood pressure in general, including to the legs, if the dog was at rest; but that sympathetic stimulation was accompanied by *dilated* blood vessels and increased blood flow if a limb was exercising.

Victor's own experiments showed the same mechanisms seemed to be operating in exercising versus resting human subjects. "If you're exercising on a stationary bike, holding on with your arms, the body wants to constrict blood flow to the arms because that helps keep blood pressure up so you don't pass out when you exercise," Victor explains. "But at the same

time, blood flow has to be directed to your exercising legs, because those muscles need oxygen from the blood."

'Dilator' chemicals released in muscles

In the 1980s, Victor was part of a research team that developed an accurate, noninvasive way to measure sympathetic nervous system activity via surface electrodes.

"It turned out to be a wonderful technique to understand how the skeletal muscle blood flow is regulated during exercise," he says.

In 1986, Victor joined the faculty at the University of Texas Southwestern Medical School in Dallas, where he continued his blood flow research until moving to Cedars-Sinai in 2009.

"We started with rat studies," he says of blood flow and exercise studies conducted in the early 1990s in Dallas. "In exercising skeletal muscle, it was becoming clear that the sympathetic nerve fibers lose their ability to constrict the blood flow because the working muscles are releasing some dilator chemicals. We wanted to figure out what those chemicals were."

The main one, the team found, was nitric oxide, also known as NO, a molecule that was to get a great deal of attention by the end of the decade. NO, it was soon learned, is a gas synthesized by an enzyme called nitric oxide synthase, or NOS. When extra blood flow is needed, NOS produces NO, which signals blood vessels to dilate.

NOS, NO, erections and exercise

NOS and NO are not only important for exercise but are essential to penile erection. Understanding this was the basis for the development of Viagra (sildenafil), first marketed in the United States in 1998 and followed by similar drugs in the "PDE5 inhibitor" class. PDE5 inhibitors prolong the vasodilating effects of NO by interfering with its normal breakdown.

At the same time that the actions of NOS and NO were being studied by researchers in erectile dysfunction, they also were receiving attention from muscle biologists, such as MDA grantee Kevin Campbell at the University of Iowa and MDA-supported James Stull at UT Southwestern.

"It was really wonderful," Victor says of the 1990s research. "Jim Stull's lab was around the corner from our lab. He's a muscle biologist and I'm a cardiovascular person, and we were trying to figure out what nitric oxide was doing in muscle. We worked on the project together."

Stull and Victor bred mice missing NOS in their skeletal muscles and found they lost the ability to dilate their blood vessels in exercising muscles. "The difference between resting and exercising muscle disappeared," Victor recalls.

Without dystrophin, NOS is missing in action

It was also during the 1990s that scientists such as Campbell and many others funded by MDA made rapid progress in describing the cluster of proteins at the muscle-fiber membrane: their normal locations and functions, and the consequences of their absence.

A deficiency of the protein dystrophin, part of this multiprotein cluster, had been known since 1986 to be the underlying cause of DMD and BMD.

In the 1990s, it was found that NOS normally is tethered to this protein cluster, and that when dystrophin is missing, NOS isn't in its proper position either.

The skeletal muscle form of NOS is called "neuronal" NOS, or nNOS. It's also present in the nervous system.

Victor and his colleagues speculated that at least some of the fatigue, exercise intolerance and muscle damage seen in dystrophin-deficient mice and in patients with DMD and BMD might stem from one of the secondary effects of dystrophin deficiency: loss of nNOS at the muscle-fiber membrane.

Without nNOS at the membrane, they surmised, NO was probably likewise deficient, and therefore the normally expected vasodilation and increase in blood flow to exercising muscles wasn't happening.

Not enough blood flows to exercising muscles

With Stull and several other researchers, Victor published a paper in 1998 showing that mdx mice (a model of human DMD) shared something with the nNOS-deficient mice they had studied: They lacked the ability to counteract sympathetic stimulation and couldn't dilate their muscle blood vessels during exercise.

"We followed it up quickly with a parallel clinical study in boys with Duchenne dystrophy," Victor says. They received help from Susan Iannaccone in Dallas, who had stored muscle biopsy tissue from many patients with DMD. (Iannaccone, a pediatric neurologist, has received MDA research support and is currently director of the MDA Clinic at Children's Medical Center of Dallas.)

With Iannaccone, Stull, and others, Victor showed in 2000 that boys with DMD experienced the same impairment of vasodilation in skeletal muscles in response to exercise as was seen in nNOS-deficient and mdx mice.

Boys of the same age who had other muscle diseases in which nNOS was properly localized had normal exercise-related vasodilation.

Can a NO boost restore blood flow?

"We thought about blocking the sympathetic nervous system," says Victor, as a way of restoring vasodilation in exercising muscles in DMD. "But you have to do that locally. You can't just block the sympathetic nerve fibers generally, or blood pressure would drop."

If NO were completely absent in skeletal muscle because of mislocalized nNOS at the membrane, then trying to prolong NO's activity using a Viagra-like drug wouldn't do any good, Victor reasoned. But if there were some NO being produced, then such a strategy could be considered.

Additional work would show that there is, in fact, some NO being produced in people with DMD or BMD, and that its longevity could therefore perhaps be increased by treating patients with a PDE5 inhibitor.

Testing tadalafil

In 2010, Victor received an MDA grant to study the effect of tadalafil (Cialis, a Viagra-like drug) on blood flow in exercising forearm muscles in men with BMD.

"We've started very conservatively with one dose of tadalafil," Victor says. "If the study is positive, the next step is to reduce the dose and find what the lowest effective dose is. If the study looks negative, we want to ask the FDA (U.S. Food and Drug Administration) for approval to give the drug ever day for a week."

Asked about possible side effects, including the potential for harmful, prolonged erections, Victor said, "I would not necessarily view this as a chronic therapy. It could be given before exercise. Let's say a patient knew he wanted to exercise on Monday. He could take the drug ahead of time and it might allow more exercise without muscle injury.

"The gratifying part of this has been the parallel findings in rats, mice and patients. I think the key question is: Will the clinical dosing be enough? That's where the rubber hits the road. For now, the animal experiments and the human experiments are all up and running. We're moving forward."

Participants will do handgrip exercise

To find out about the tadalafil in BMD study at Cedars-Sinai, contact Dominique Durant in Los Angeles at (310) 248-8080 or Julie Groth at (310) 248-7641.

Diverting an Unwanted Protein

John Knopf, Ph.D.

Affiliations:
Acceleron Pharma, Cambridge, Mass.

Strategy:
Inhibiting myostatin and other limiters of muscle growth and regeneration

Status:
Clinical trial

When John Knopf co-founded Acceleron Pharma in the spring of 2003, a muscle protein called myostatin had been on his and other researchers' radar for several years.

The protein had been identified as a "negative regulator" (limiter) of muscle growth and regeneration back in 1997. And, since that time, myostatin-deficient mice and cattle had been shown to have large, strong muscles without any apparent ill effects.

Additionally, two key research papers had been published in 2002, both of which had direct relevance to muscular dystrophy research. They reported experiments conducted in mice lacking a protein called dystrophin and showing a disease resembling human Duchenne muscular dystrophy (DMD).

One set of experiments involved knocking out myostatin genes in dystrophin-deficient (mdx) mice; the other involved mdx mice that were given antibodies (immune system proteins) that blocked myostatin protein activity.

The mdx mice bred not to produce myostatin were stronger and more muscular than their mdx counterparts with normal myostatin levels. And, the mdx mice treated with anti-myostatin antibodies for three months showed more muscle mass and muscle strength, as well as a significant decrease in muscle degeneration, compared with their untreated counterparts.

The combined effects of these findings paved the way for further research and development of myostatin-inhibiting therapies as a potential treatment of DMD and perhaps other muscular dystrophies — and that's what Knopf and his colleagues at Acceleron wanted to do.

John Knopf

Early research used myostatin antibody

By 2003, Knopf, who has a doctorate in molecular and cellular biology from the State University of New York at Buffalo, had been working in the biotechnology industry for several years. His most recent position had been in the research division of Wyeth, a pharmaceutical company that's now part of Pfizer.

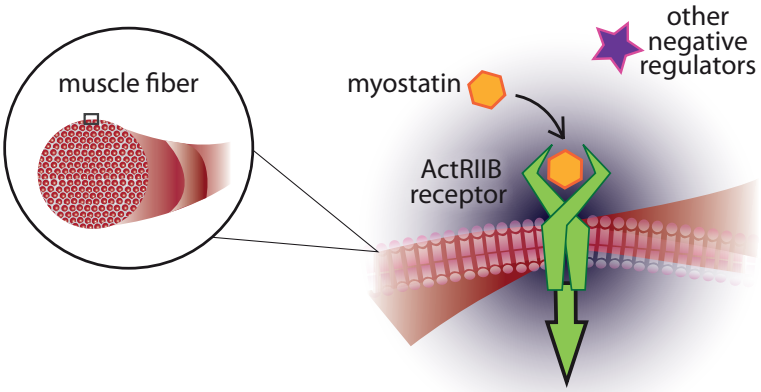
Wyeth also became interested in blocking myostatin, especially after 2004. In that year, news about a healthy, large-muscled child with a genetic myostatin deficiency reached the world via a paper in the New England Journal of Medicine, igniting the field. The 4-year-old boy, identified in Germany, had almost no myostatin, had large, strong muscles, and had no apparent health problems.

Wyeth developed an antibody to myostatin and began testing it in 2005 in adults with a variety of muscular dystrophies. It would prove to be safe but not beneficial.

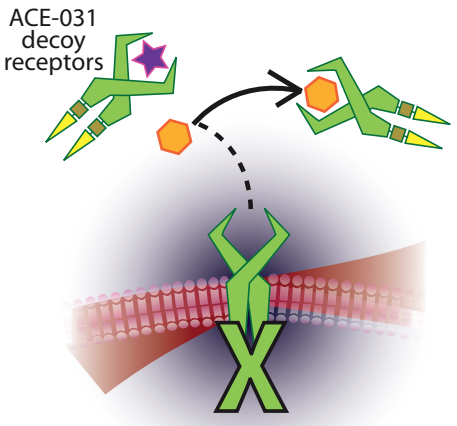
"We don't know much about the Wyeth studies," says Knopf, who had left Wyeth by the time the company got seriously interested in the subject, "but that's a big question we get: 'Didn't folks already try to inhibit myostatin, and didn't that fail?'"

Knopf answers that question by emphasizing that the apparent lack of benefit from the

Mechanism of ACE-031



Normally, myostatin and other negative regulators of muscle dock on receptors called ActRIIB on the muscle-fiber membrane surface. They send signals through these receptors that limit muscle growth and regeneration.



ACE-031 is designed to be a circulating "decoy" ActRIIB receptor, capable of trapping myostatin and other negative muscle regulators and diverting them from the surface receptors through which they would normally send their growth-limiting signals.

anti-myostatin antibody therapy does not mean that inhibiting myostatin and other proteins using a different approach would not be effective.

In fact, he says, data from mouse studies suggest that the "decoy receptor" strategy Acceleron has chosen is much more promising than the antibody strategy.

'Decoy receptor' binds myostatin

"Myostatin," Knopf explains, "binds to a receptor (docking site) that's present on the surface of cells. When it binds to that receptor, it tells the body to make less muscle." It's that interaction of myostatin with a receptor called ActRIIB that Knopf and his colleagues at Acceleron want to interrupt.

Knopf's team created a decoy ActRIIB receptor, one that dissolves in blood and can bind circulating myostatin. Myostatin stuck to this decoy is unable to bind to its naturally occurring receptors, and it's diverted from its normal role, which is signaling muscle to stop growing or regenerating.

Knopf also points out that there are other proteins related to myostatin that normally stick to ActRIIB receptors and that these too limit muscle growth, development and regeneration in different ways and at different time points.

"The idea is to inhibit several of these proteins at once with one decoy," Knopf says, "with the potential not only to increase the muscle mass, but also to affect the overall quality of the muscles."

ACE-031 now being tested in boys with DMD

Acceleron recently began testing their soluble form of ActRIIB, called ACE-031, in boys with DMD in Canada. In September 2010, Acceleron (www.acceleronpharma.com) entered a collaboration with the specialty biopharmaceutical company Shire (www.shire.com) in which the companies will jointly collaborate on a worldwide development program to advance ACE-031 into a global phase 2/3 clinical program designed to demonstrate long-term disease modification in DMD patients.

In January 2011, MDA awarded a $1.5 million grant to Acceleron to support and expand the ongoing clinical studies of ACE-031 in boys with DMD.

"We're optimistic that indeed we'll show some efficacy for muscular dystrophy," says Knopf. "We're hoping for stronger muscles and muscles that are less susceptible to damage — which is the opposite of what you get in muscular dystrophy, where you see weakening muscles that are more susceptible to damage. We hope to reverse that course." ❑

For information about the ACE-031 trials in Canada, contact Rhiannon Taranik at (519) 685-8441 or Rhiannon.Taranik@lhsc.on.ca, or send email to clinicaltrials@acceleronpharma.com.

Designing a Better Gene

Dongsheng Duan, Ph.D.

Affiliation:
University of Missouri, Columbia, MO

Strategy:
Restoring nNos to the muscle-fiber membrane

Status:
Laboratory experiments

Dongsheng Duan's interest in gene therapy to treat diseases goes back a long way, although his initial focus wasn't muscular dystrophy.

"I came to the muscle field by accident," says Duan, an MDA research grantee and professor of molecular microbiology and immunology at the University of Missouri at Columbia. "It was not something I intended to do when I was young."

After earning a doctorate in pathology at the University of Pennsylvania in 1997, Duan joined the laboratory of John Engelhardt at the University of Iowa for his postdoctoral training. The lab was focused on gene therapy for cystic fibrosis, a genetic lung disease.

By the early 2000s, Duan was looking for a different field in which to apply his gene therapy expertise. He became interested in muscular dystrophy and in 2002, moved to the University of Missouri, where academic veterinarian Joe Kornegay had established a colony of dogs with a DMD-like disease. (Kornegay, a current and former MDA grantee, is now director of the National Center for Canine Models of DMD at the University of North Carolina at Chapel Hill.)

Almost as soon as he started working in Missouri, Duan identified a gap in muscular dystrophy research that he wanted to help fill.

What happens between gene mutation and disease symptoms?

Since the late 1980s, scientists had known that mutations in the gene for the muscle protein dystrophin were the root cause of DMD and Becker muscular dystrophy (BMD). But, Duan noticed that the steps between dystrophin mutations and the symptoms of DMD or BMD were not clearly defined.

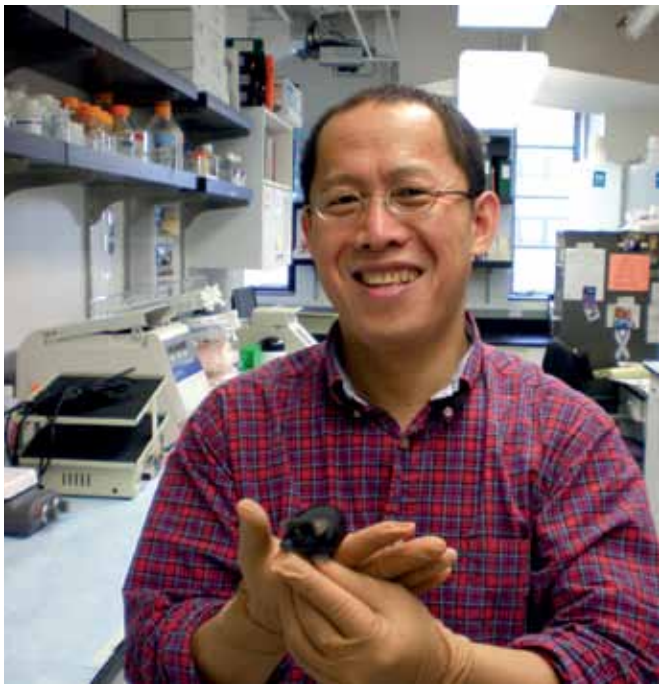
"Every time I opened a journal article, I read, 'Duchenne muscular dystrophy is caused by mutations in the dystrophin gene,'" he notes. "But from the mutation to the disease, there seemed to be a gap. The pathways were not clearly defined." To define them, Duan decided to look back in scientific history.

When his search took him all the way back to the early 1970s, Duan found that some experts at the time had reported impaired blood flow — ischemia — as a possible factor in DMD.

Most of that work had been forgotten by the 1990s, after the dystrophin gene had been identified and the majority of scientists had shifted their focus to the protein's crucial role in protecting the muscle-fiber membrane. Duan wondered whether dystrophin's absence could account for the ischemia noted by those researchers many years earlier.

"When I moved through history into 1995, there were papers showing that one of dystrophin's roles is to recruit neuronal nitric oxide synthase [nNOS] to the muscle-fiber membrane," he says. The nNOS enzyme makes nitric oxide (NO), a protein that opens up (dilates) blood vessels. Although it's called "neuronal," it's also found in muscle fibers. Could it be, Duan reasoned, that lack of dystrophin could lead to lack of nNOS, lack of NO, and ultimately, lack of blood-vessel dilation with exercise?

Things really clicked for Duan when he read a 1998 paper showing that dystrophin-deficient mdx mice, which, like humans with DMD, lack nNOS at the muscle-fiber membrane, have impaired blood flow in exercising muscles. (For more, see page 6.) Maybe some of the damage in muscles affected by DMD and BMD was because of ischemia after all.



Dongsheng Duan

"This kind of all added up," Duan recalls. "When I went back and looked at my own samples, which we had collected from dystrophic dogs and dystrophic mice, what was really striking to me was that not every muscle fiber is injured, even though they all lack dystrophin. You get a kind of focal lesion. It's not like the entire muscle is damaged. That's similar to a situation where you have ischemia, where you get locations where there wasn't enough perfusion of blood. I thought all this probably had something to do with the function of nNOS."

Utilizing minidystrophin genes

Dystrophin, a very large gene, has to be miniaturized to be given as gene therapy. When it's miniaturized, sections of its DNA are removed, resulting in production of a less-than-full-length dystrophin protein molecule.

Duan suspected — and later showed — that the miniaturized dystrophin genes (known as minigenes and microgenes) that researchers had developed since the 1990s did not contain the sections of DNA that would allow the dystrophin protein to stick to nNOS. Therefore, he speculated, they might only be fixing some of the problems that stem from dystrophin deficiency.

"We made two types of transgenic mice," he recalls. "Both expressed minidystrophin genes. They had identical structures except one had sections called R16 and R17, which carry the code for the nNOS-binding part of the protein. The other one did not have those sections. We looked at muscle function to see if there was a difference between them. If nNOS meant something, I reasoned, we should see a difference."

The experiments found that the mice with a minidystrophin gene missing sections R16 and R17 could not restore NOS to the muscle-fiber membrane and showed reduced blood flow and ischemic damage in their muscles, while the mice with the slightly longer gene that contained these sections had normal blood flow and no ischemic damage.

In later experiments, Duan and his co-workers put the two types of mice on a treadmill. "On the first day, both groups performed the same," he says. "But after that, muscle function in the group with the smaller gene and no nNOS binding started going down. By the time we got to day six or eight, we saw a big difference. We looked at sections of the muscle to find out if ischemic damage was occurring in the mice that did not restore nNOS, and we found it.

"That clearly provided evidence for saying that if you don't have nNOS at the membrane, there is going to be ischemic damage to the muscle fiber. That's definitely going to affect function, such as walking ability."

Duan says the findings have important implications for therapies being developed for DMD and BMD and perhaps even for other forms of muscular dystrophy, noting that nNOS may be mislocalized in some other muscular dystrophies as well.

"If you have genes that restore nNOS and some that don't restore nNOS to the membrane, you want to choose the ones that restore nNOS," he says.

In addition, he notes, "you may want to find other ways, such as vasodilating medications, to help patients open their blood vessels when they exercise. As we develop therapies that help people with muscular dystrophy become healthier and lead more normal lives, they'll want to exercise."

Resynchronizing the Muscle Ballet

Eric Hoffman, Ph.D.

Affiliations:

Children's National Medical Center, Washington, D.C.
George Washington University, Washington, D.C.
Validus Biopharma, Rockville, MD

Strategy:

Coordinating degeneration and regeneration signals in muscle

Status:

Drug development

"I was always interested in the application of genetics to human problems," says Eric Hoffman, a molecular geneticist and MDA research grantee who directs the Center for Genetic Medicine Research at Children's National Medical Center in Washington.

In the mid-1980s, as he was finishing his doctorate in biology at Johns Hopkins University, Hoffman says, "I began looking around for a human disease that seemed to be on the cusp of making progress, and Duchenne muscular dystrophy seemed to be number one."

In 1986, Hoffman moved to Children's Hospital and Harvard Medical School in Boston to begin a postdoctoral fellowship with Louis Kunkel, then a new researcher in Duchenne muscular dystrophy (DMD) who had a grant from MDA to study the disease. Later that same year, Kunkel and Hoffman (armed with his own MDA grant) and others identified mutations in the gene for a previously unknown muscle protein as the underlying cause of DMD.

The muscle protein would soon be known as "dystrophin."

Hoffman and Kunkel have remained major figures in DMD research ever since, and both have continued to receive MDA support. Kunkel has remained at Harvard, while Hoffman moved from Harvard to the University of Pittsburgh in 1990 and then to Children's National and George Washington University in Washington in 1999.

Beyond genetic mutations

The dystrophin findings were no doubt among the most important advances in DMD made since the first descriptions of the disease more than a century earlier. But by the late 1990s, Hoffman was looking beyond the mutations themselves.

"Historically, the focus was on the primary gene defect," he says. "People talk about a genetic mutation as 'ground truth,' and in many respects, it is. But the primary gene defect is not the be-all and end-all to everything. It's the initiation of a process, and it's the process — all the things that happen 'downstream' of the genetic defect — that really affects the patient."

Uncoordinated cycles of degeneration and regeneration

These days, what interests Hoffman most among the many downstream effects of dystrophin deficiency in muscle tissue

Eric Hoffman

are the chronic and apparently uncoordinated cycles of muscle degeneration and regeneration.

Normally, Hoffman says, an injured muscle fiber goes through degeneration and regeneration as part of an orderly process that takes about two weeks.

"Everything has its time and place," he explains. "Macrophages [the immune system's first responders] should come in, do their cleanup work in a day or so, then activate muscle stem cells, and then leave, because their job is done.

"Everything is this nicely coordinated ballet stretches over two weeks, with cells coming and going, talking to each other, and then leaving."

Chronic pathology, he says, results from "having a conductor start the orchestra for a ballet and then start it over again every 15 minutes, without telling the group of dancers already on the stage to leave. In Duchenne dystrophy, you can have one region of a muscle that's in day four of the degeneration-regeneration process, a neighboring region that's in day seven, and another neighboring region that's in day one."

Unfortunately, chemical signals released from cells involved in each "ballet" cross the boundaries between fibers, sometimes restarting a dance sequence that should be nearing completion, sometimes prematurely terminating one that's just begun.

Resynchronizing cycles of degeneration and regeneration

Hoffman thinks the action of prednisone — a corticosteroid drug that's widely used to treat DMD — may help with this "uncoordinated ballet," but in a way that's different from what most people think.

The traditional way of looking at prednisone, he notes, is that it's a potent anti-inflammatory drug. That may be so, Hoffman says, but prednisone may not be directly involved in shutting off inflammation. Instead, it may be doing so indirectly, by resynchronizing the degeneration-regeneration ballet.

Prednisone, he notes, is derived from cortisol, a hormone secreted by the adrenal glands. Cortisol is a master timekeeper, coursing through the circulation at 3 or 4 a.m. and nearly gone by midafternoon every day, its waxing and waning concentrations influencing the timing of many biological events.

Cortisol and its derivatives, such as prednisone, appear to "loudly tap the conductor's podium," Hoffman says, telling the performers, "We're starting again. Everyone back to your original places."

The other effects of cortisol and prednisone are related to their ability to switch genes on and off in cell nuclei, with a variety of consequences. This gene switching, which some experts think of as the primary beneficial effect of prednisone, is to Hoffman's way of thinking the primary culprit behind unwanted side effects such as weight gain, bone thinning and cataracts.

Modifying corticosteroids

In 2008, Hoffman started Validus Biopharma with medicinal biochemist John McCall and muscle inflammation expert Kanneboyina Nagaraju. The goal of this small company is to develop modified corticosteroids that can resynchronize degeneration and regeneration cycles in muscle and other tissues without doing the other things that these drugs do, because they don't switch genes on and off.

(Validus has a grant from MDA Venture Philanthropy, the drug development arm of MDA's translational research program. Hoffman has additional MDA support to study the mechanism by which corticosteroids act in DMD.)

"We've shown that Validus Biopharma drugs can resynchronize tissue remodeling," Hoffman says, "so you don't have the poor cross-talk with the nasty wrong signals. The signals aren't running into each other as much. They're coordinated. If you give them to dystrophin-deficient mice, you get rid of all the inflammation, but you don't see the side effects of prednisone."

Hoffman says he hopes Validus will be able to test one of its modified corticosteroids in patients with DMD in 2012.