On the Path to a Duchenne Muscular Dystrophy Therapy

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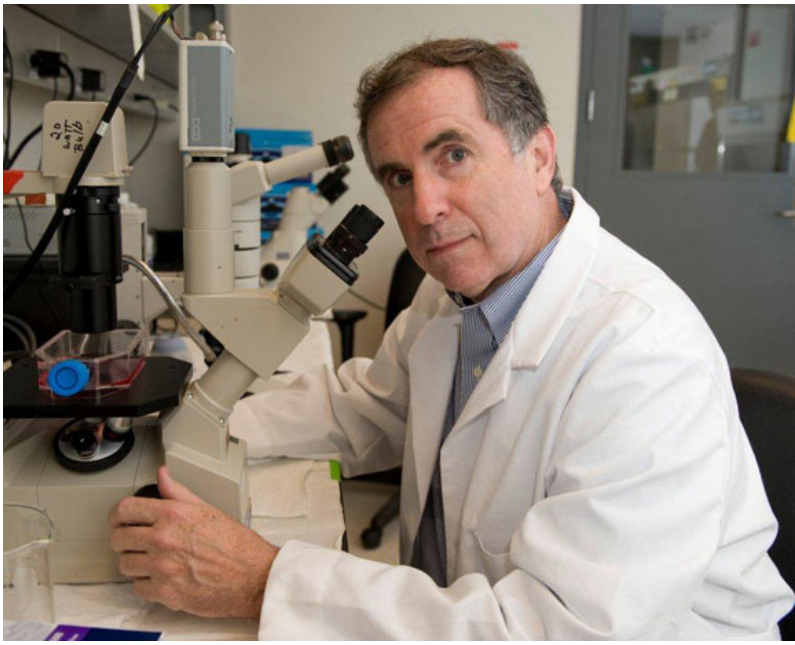
ABSTRACT

Duchenne Muscular Dystrophy (DMD) is a devastating inherited disease of children with no effective therapies. Here I discuss the landscape for new treatments and the history, current status and prospects for our work developing recombinant biglycan as DMD therapy.

KEYWORDS: Biglycan, Duchenne Muscular Dystrophy, neuromuscular disorders



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Justin Fallon in his lab at Brown University.

INTRODUCTION

Duchenne Muscular Dystrophy (DMD) is the most common form of muscular dystrophy. DMD is caused by mutations in dystrophin, a protein that is essential for maintaining the integrity of both cardiac and muscle cells (Emery, 2002; Nowak and Davies, 2004). Starting at about age four, the affected boys exhibit muscle weakness and most are in wheelchairs by their teens. Death is usually caused by failure of the diaphragm and/or cardiomyopathy. Patients rarely survive past their mid-twenties. Effective treatments for this devastating disease are urgently needed.

Current therapies for muscular dystrophies are not disease-modifying and have limited impact on the clinical outcome. The current standard of care is steroids, either prednisone (Mendell et al., 1989) or Deflazacort (a synthetic prednisolone; Biggar et al., 2004). These agents impede inflammatory fibrosis and improve muscle strength. Unfortunately, after an initial increase in strength in the first six months to one year, patients on these medications often exhibit a slow decline after 18 months (Griggs et al., 1991). Both these drugs have significant side effects that can limit their use. Physical rehabilitation, including stretching exercises, can maintain greater flexibility in muscles susceptible to contracture formation. However, most methods of rehabilitation become ineffective once the disease reaches its greatest severity by the second decade of life.

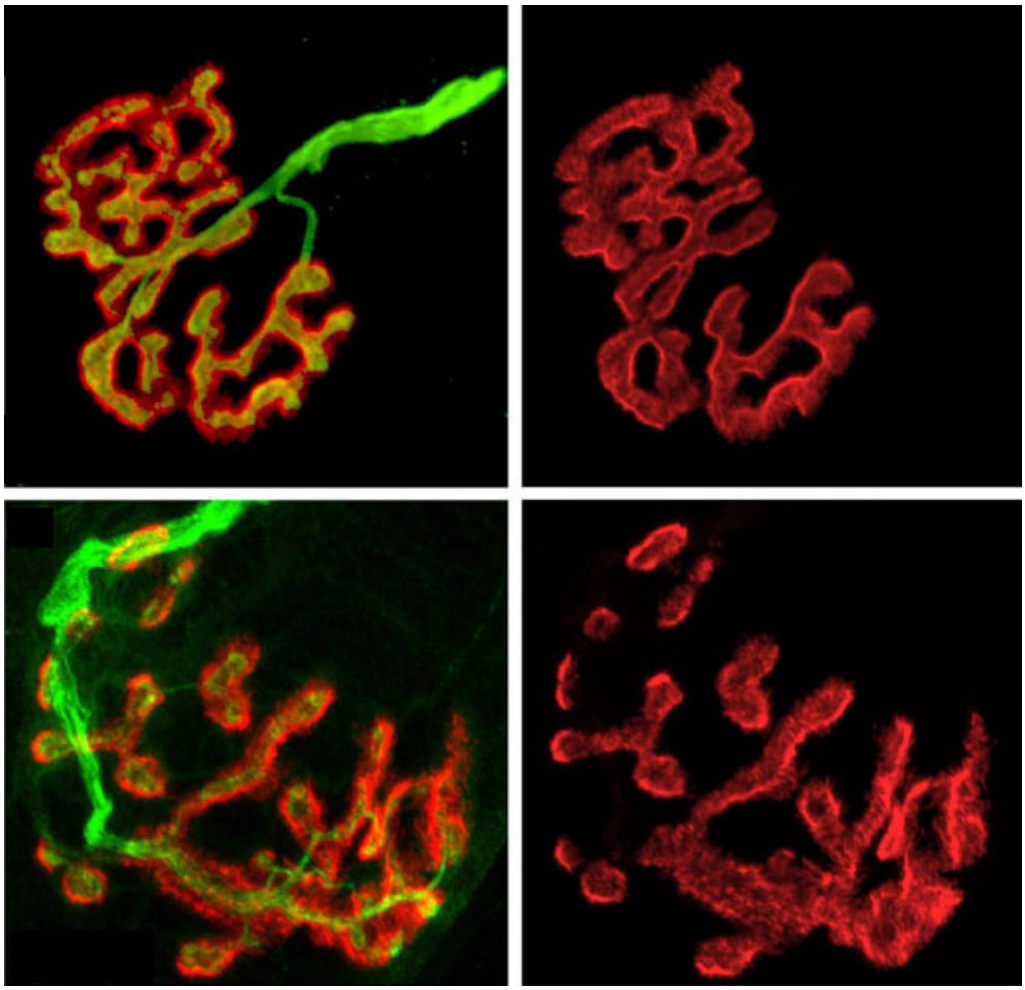
The good news is that a wide range of DMD therapeutic strategies are under investigation, with some promising compounds in late-stage clinical trials. Gene therapies seek to replace, repair or override the mutated dystrophin gene. The most advanced of this class employ a dystrophin minigene delivered by adeno-associated viral vectors (Blankinship et al., 2006). Since muscle is a regenerative tissue, cell-based therapies have drawn much attention. However, it has been difficult to achieve sufficient engraftment of the transplanted cells. Recent approaches using mesangioblasts in mouse models have started to break down this barrier (Sampaolesi et al., 2003), but human studies are still in the planning stages.

Pharmacological interventions include small molecule drugs to induce stop codon read through, which would be effective for ~15% of DMD patients. Ataluran, whose development was inspired by the observation that gentamicin has such activity (Wagner et al., 2001), has been tested in a large clinical trial (PTC Therapeutics). Other pharmacological therapies are also being pursued that either improve muscle performance or mitigate the pathological process in DMD muscle. These include using humanized antibody fusion proteins that neutralize myostatin and related inhibitors of muscle growth. Unfortunately, this class of compounds has not yet proven effective in clinical trials.

Finally, treatments aimed at reducing muscle fibrosis are also in development. Since scarring interferes with the function of remaining normal muscle and degrades the stem cell niches necessary for regeneration, such treatments

could confer significant benefit to the patients.

Exon skipping is an exciting new approach that employs synthetic oligonucleotides to excise selected regions of the mutated dystrophin mRNA by regulating alternative splicing (Cirak et al., 2011). The product is a 'Becker-like' dystrophin protein that, while truncated, harbors activity that would be expected to confer significant benefit to patients. Early small-scale studies in humans targeting exon 51 using two different oligonucleotide chemistries have yielded encouraging results and late-stage trials are currently underway (drisapersen and eteplirsen; GlaxoSmithKline and Sarepta, respectively). Both studies have shown that the expression of (truncated) dystrophin is restored in a subset of muscle fibers. Most importantly, the subjects have maintained ambulation to a remarkable degree when compared to historical controls. These compounds are a leading example of personalized medicine, since the therapy is tailored to specific mutations. However, since mutations can occur virtually anywhere in the very large dystrophin gene, a given compound can only target a subset of patients. For example, exon 51-targeted oligonucleotides could benefit about 15% of patients. With current methodology it is estimated that additional compounds could be developed that would collectively target about 50% of patients. A further limitation of exon skipping is that none of the current oligonucleotide chemistries target the heart.



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Segmented synapses

Synaptic structures in mice engineered to lack the protein biglycan (bottom row) appear discontinuous compared to the synaptic structures in normal mice (top).

One of the most long-standing and appealing pharmacological approaches to treat DMD is the upregulation of utrophin, an autosomal homolog of dystrophin (Khurana and Davies, 2003). Utrophin is normally expressed at high levels during fetal development and in early childhood, but in the mature animal it is restricted to the neuromuscular and myotendinous junctions. The high levels of utrophin in young children is likely one of the reasons that the clinical manifestations of DMD only appear after about 4 years of age. Genetic studies have shown that if utrophin is up-regulated it can functionally replace dystrophin in mdx mice (Tinsley et al., 1998). Muscle death is prevented and muscle function is restored to wild-type levels. A utrophin-targeted approach is also appealing since it targets an endogenous, fetal program that could compensate for the loss of dystrophin. Finally, a utrophin-based therapy should target all DMD patients, regardless of mutation.

THE PATH TO BIGLYCAN AS A DMD THERAPEUTIC

Our laboratory is developing a recombinant form of the endogenous extracellular matrix protein biglycan as a DMD therapeutic. Although I did not know it at the time, this idea can be traced back to when I was a postdoctoral fellow with U.J. McMahan at Stanford. The goal of these studies was to identify and characterize the proteins that organize the muscle cell membrane at the synapse. We discovered agrin, an extracellular matrix protein that organizes acetylcholine receptors into discrete domains on the muscle cell surface (Fallon et al., 1985; Nitkin et al., 1987). In my own laboratory

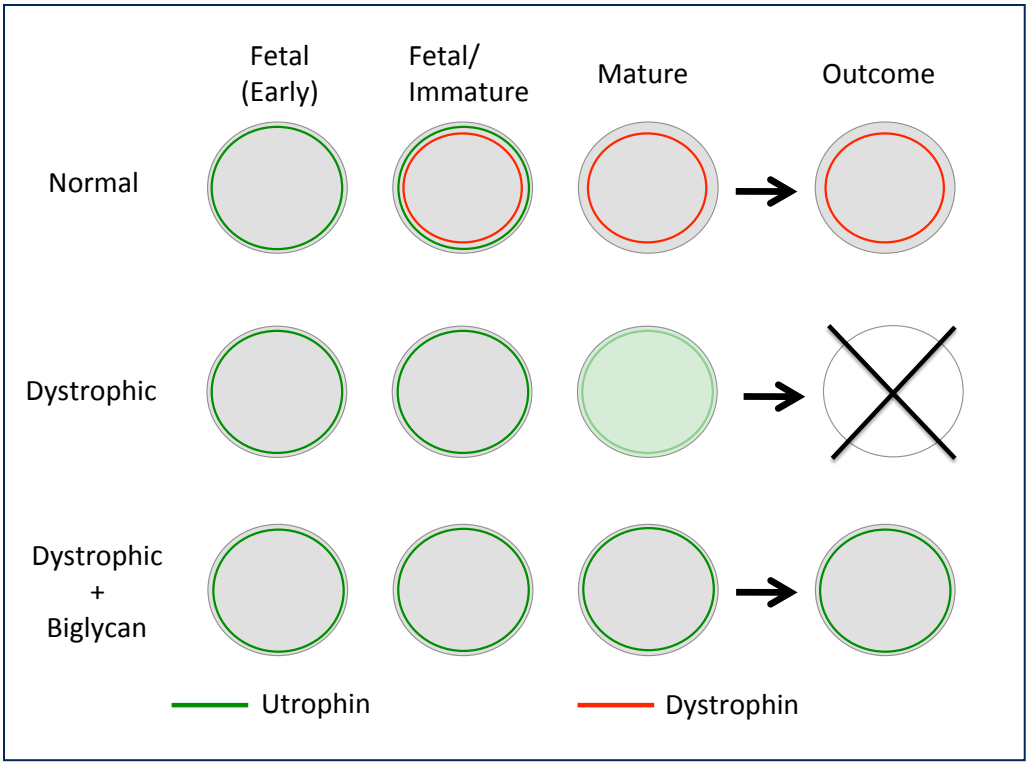

Figure 1. Rationale for Utrophin-directed DMD therapy.
In normal muscle utrophin is highly expressed during development, but then is down-regulated and replaced by dystrophin as the muscle matures. In boys with DMD there is no dystrophin and the levels of utrophin are insufficient to maintain muscle health. Shown are schematic cross-sections of myofibers depicting the distribution of utrophin and dystrophin in normal individuals, DMD patients and proposed therapeutic benefit of delivering recombinant biglycan to upregulate utrophin in dystrophic muscle. Red: dystrophin; Green: utrophin. See text and Amenta et al., 2011 for details.

we went after the cell surface proteins that bind agrin and mediate its activity. This effort led to dystroglycan, which had just been found to be a key member of the complex of proteins that associate with dystrophin (Bowe et al., 1994). Utrophin was known to be at the neuromuscular junction – even in DMD. We began to wonder whether we had tapped into a mechanism that regulates utrophin expression. If so, we realized that it might be possible to harness this pathway to create a new DMD treatment.

We sought to explore this new hypothetical pathway. This work was carried out in my laboratory at Brown University. I have been incredibly fortunate to work with a team of remarkably talented and dedicated scientists. These include Mark Bowe, Katherine Deyst, Mike Rafii, Hiroki Hagiwara, Mary Lynn Mercado, Beatrice Lechner, Sarah Mentzer, Carolyn Schmiedel and Alison Amenta. An especially important member of the team is Beth McKechnie, who has been on this project for over 20 years and has contributed many of the key insights that have brought us to the cusp of clinical trials.

The first clues came from biochemistry; we looked for additional dystroglycan binding proteins and discovered biglycan in this complex (Bowe et al., 2000). Continuing down this biochemical path, we found that biglycan also binds to alpha and gamma sarcoglycans (Rafii et al., 2006). This result was exciting because these sarcoglycans are only found in the two tissues affected by DMD: skeletal muscle and heart. We went on to investigate biglycan function using mutant mice created by Marian Young at the NIH (Young and Fallon, 2012). These studies yielded the critical information that biglycan is important for the proper expression of several dystrophin-associated proteins such as the sarcoglycans and an intracellular signaling complex including nNOS (neuronal nitric oxide synthase; Mercado et al., 2006). However, from a DMD therapy viewpoint, the critical finding was that biglycan also regulates utrophin in early development (Amenta et al., 2011). We now had a link to a potential therapeutic pathway.

The transition from an idea to a viable therapeutic is complex and lengthy. However, the first question is simple – can we produce a candidate compound and show that it can be delivered in a form, route, dose and frequency that is amenable to use as a drug? We therefore produced recombinant biglycan (rhBGN) and asked if it was active in mouse models of DMD. Remarkably, systemically-delivered rhBGN up-regulated utrophin at the muscle membrane and improved the health and function of the dystrophic muscle (Amenta et al., 2011). Equally important, rhBGN was active at doses (2-10mg/kg) and frequencies (once injection every two weeks) that are suitable for use in patients.

With these first results in hand we began a concerted effort to bring rhBGN to clinical trials. These efforts require expertise beyond that of an academic laboratory. Therefore I cofounded Tivorsan Pharmaceuticals, a Rhode Island-based company committed to develop rhBGN as a therapeutic for DMD (www.tivorsan.com). Tivorsan has marshalled the necessary regulatory, manufacturing and clinical expertise that will be needed to complete preclinical work and initiate clinical testing.

FUTURE DIRECTIONS

Biglycan could have therapeutic benefit in amyotrophic lateral sclerosis (ALS). ALS is a neurodegenerative disease marked by the loss of upper and lower motor neurons (Pasinelli and Brown, 2006). However, the first sign of pathology is destabilization of the nerve-muscle synapse, resulting in deafferentation and muscle paralysis. A therapy that stabilizes this synapse could thus prolong function in ALS patients. As discussed above, our path to biglycan stemmed from an inquiry into the how nerve-muscle synapses are formed. In an exciting recent finding we showed that biglycan binds to the receptor tyrosine kinase MuSK, the central organizer of this synapse. Further, biglycan is important for synapse stability. These basic science findings raise the possibility that rhBGN could stabilize the compromised synapses in ALS patients and delay the progress of the disease. Experiments to test this idea in mouse models of ALS are underway in the laboratory. If these studies in model organisms are favorable, we will be well positioned to initiate testing of rhBGN in ALS patients.

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