Duchenne muscular dystrophy is ultimately a stem cell disease

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For years, scientists have tried to understand why children with Duchenne muscular dystrophy experience severe muscle wasting and eventual death. After all, laboratory mice with the same mutation that causes the disease in humans display only a slight weakness. Now research by scientists at the Stanford University School of Medicine, and a new animal model of the disease they developed, points a finger squarely at the inability of human muscle stem cells to keep up with the ongoing damage caused by the disorder.

"Patients with muscular dystrophy experience chronic muscle damage, which initiates a never-ending cycle of repair and wasting," said Helen Blau, PhD, the Donald E. and Delia B. Baxter Professor and a member of Stanford's Institute for Stem Cell Biology and Regenerative Medicine. "We found that in mice the muscle stem cells can keep up with the demands on them to cycle."

The difference is caused, the researchers found, by the fact that mice have significantly longer protective caps on the ends of their chromosomes. The caps, called telomeres, allow the cells to continue to divide and replenish the damaged muscle long after the human cells have reached their capacity for division.

The research marks the first time that muscular dystrophy has been shown definitively to be a stem-cell-based disorder, according to the scientists, who also generated the first-ever mouse model of Duchenne muscular dystrophy that closely mimics the human disease. Similar to human patients, the animals exhibit severe muscle weakness and shortened life span. The mouse model will allow clinicians and researchers to better study the disease and test new therapies.

"The results suggest that treatments directed solely at the muscle fiber will not suffice and could even exacerbate the disease. The muscle stem cells must be taken into consideration," said Blau. Former postdoctoral fellow Jason Pomerantz, MD, co-corresponding author and now an assistant professor at the University of California-San Francisco, said, "if a treatment does not replenish the stem cell compartment, it will likely fail; it would be like pushing the gas pedal to the floor when there is no reserve."

Blau is the senior author of the research, which will be published online Dec. 9 in *Cell*. Postdoctoral scholars Alessandra Sacco, PhD, and Foteini Mourkioti, PhD, are co-first authors of the work. Sacco is now an assistant professor at the Sanford-Burnham Medical Research Institute.

Duchenne muscular dystrophy is the most prevalent form of the muscular dystrophies. It is caused by a mutation in the dystrophin gene, which connects the interior cytoskeleton of the muscle fiber to the extracellular matrix. Its absence leads to death of the muscle tissue and progressive weakness, which eventually affects a patient's ability to breathe; 10-year-olds are often wheelchair-bound. Death usually occurs by the second or third decade as a result of respiratory and heart problems. The disorder affects about one of every 3,500 boys in the United States, whereas girls are generally spared because the gene lies on the X-chromosome.

Unfortunately, for decades the trusty laboratory mouse failed scientists trying to study the disease in animals. Mice with the same mutation showed only minimal muscle weakness. This left researchers without an easy way to test drugs and therapies. It also gave them a puzzle: Why were the mice so resistant to the muscle damaged caused by the dystrophin mutation?

Blau, Pomerantz, Sacco and Mourkioti, thought the answer might lie in the muscle stem cells. Like other types of stem cells, the muscle stem cells can divide to both replenish themselves and to make new muscle cell precursors. These precursor cells

can replace damaged or dead muscle cells that make up the muscle fiber. But even muscle stem cells have their limits, and in this case, the mouse cells outperform their human counterparts.

The reason, the Stanford researchers found, is in the length of the telomeres on the DNA of the two species. The average length of telomeres in laboratory mice is greater than 40 kilobases; in humans it's about 5 to 15 kilobases. Telomeres serve as protective caps on the ends of chromosomes, buffering them from the gradual shortening that occurs during each round of replication. When the telomeres become too short, the cells are no longer able to divide.

To test their theory, the researchers blocked the expression of a component of the telomerase enzyme, which maintains telomeric DNA. Mice with both the dystrophin mutation and the faulty telomerase expression experienced progressive, debilitating muscle degeneration with age - as exhibited by treadmill stamina tests and muscle damage assays - and had shorter-than-normal life spans. Muscle stem cells from the mice also had a reduced ability to proliferate, both in the animals and in culture, and were less able to engraft and begin growing when transplanted into wild-type animals.

"What we're seeing is that muscular dystrophy is a multi-factorial disease," said Blau. "The lack of dystrophin causes muscle damage. These damaged muscles are replaced by dividing muscle stem cells, but the repeated rounds of division cause the telomeres to shorten until the stem cells can't fix the damage anymore. This is what happens in humans, and in our new mouse model."

The idea that the symptoms of muscular dystrophy reflect an inability of stem cells to repair ongoing damage has some interesting implications. It implies that any successful treatment should begin early, before the stem cell pool is depleted. It also indicates that researchers and clinicians should investigate stem-cell-based therapies as well as those aimed at protecting the muscle fibers themselves. Finally, it suggests that a highly targeted approach to increase telomerase activity in the muscle stem cells could be useful.

"Finding out that this is a stem cell defect is really exciting," said Blau. "In the early 1980s we reported that muscle cells from DMD patients had less capacity to divide but we did not have the tools to figure out why, since muscle stem cells, the dystrophin gene and telomere function had yet to be identified. Finally, now we can get a handle on what is going on, and learn how best to target future therapies. Having a mouse model that mimics the human disease will benefit all in the field and is very exciting for patients."

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