New mouse model reveals a mystery of Duchenne muscular dystrophy

July 7 2013

Children with Duchenne muscular dystrophy often die as young adults from heart and breathing complications. However, scientists have been puzzled for decades by the fact that laboratory mice bearing the same genetic mutation responsible for the disease in humans display only mild symptoms and no cardiac involvement.

Now, researchers at the Stanford University School of Medicine have developed a mouse model that accurately mimics the course of the disease in humans. The study is the first to demonstrate a molecular basis for the cardiac defect that is the primary killer of people with Duchenne muscular dystrophy. Furthermore, the study provides evidence for a potential treatment to help prolong heart function. The mouse model also will allow researchers and clinicians to test a variety of therapies for the inherited condition.

"Until now, scientists had no animal model of Duchenne muscular dystrophy that manifests the symptoms of the cardiac disease that kills children and young adults with the condition," said Helen Blau, PhD, the Donald E. and Delia B. Baxter Professor at Stanford and director of the Baxter Laboratory for Stem Cell Biology. "This has been a conundrum for three decades. We found that mice with moderately shortened telomeres and the Duchenne mutation exhibit profound cardiac defects and die at a young age, just like human patients."

Blau, who is also a member of the Stanford University Institute for Stem Cell Biology and Regenerative Medicine and a professor of

microbiology and immunology, is the senior author of the study, which will be published July 7 in *Nature Cell Biology*. Foteini Mourkioti, PhD, an instructor at the Baxter Laboratory, is the lead author of the study.

The investigators found that the reason humans suffer more serious symptoms than mice has to do with the length of the protective caps, called telomeres, on the ends of chromosomes: Mice have telomeres about 40 kilobases in length, while human telomeres range from around 5 to 15 kilobases (a kilobase is 1,000 nucleotides). When the investigators introduced a second mutation in the animals that reduced telomere length to more closely match that of humans, the animals began to display the typical symptoms of the disease, including progressive muscle weakness, enlarged hearts and significantly shortened life spans.

Duchenne muscular dystrophy is the most prevalent form of the heritable muscular dystrophies. It is caused by mutations in the dystrophin gene that inhibit the production of the dystrophin protein, which connects the inside of the muscle cell to the outside matrix. The new mouse model showed that, in the absence of the dystrophin protein, the animals' heart muscle cells accumulate stress and damage due to repetitive contraction. Early treatment of a small group of animals with antioxidants protected their heart function and prolonged their lives.

Interestingly, cells in the animals from tissues that normally express dystrophin had telomeres much shorter than those in other tissues in the body that don't rely on the protein. This discovery indicates that the lack of the protein further exacerbates telomere shrinking—a fact borne out when the researchers compared affected mice and humans.

"Telomeres in heart muscle cells from four young men who had died of Duchenne muscular dystrophy were about half the length of control samples," Mourkioti said. "They are greatly shortened."

In the context of shortened telomeres, the dystrophin mutation in the mice leads to significant impairment of cardiac function. "Essentially, the heart cannot contract well," said Mourkioti, who performed a variety of functional and imaging tests, including electrocardiograms, echocardiography and MRIs, on mice in the study. When she looked at the heart muscle cells, or cardiomyocytes, of animals with the two mutations, she found damage to the cells' energy generators, or mitochondria, along with several signs of oxidative stress. (Oxidative stress is caused by byproducts of mitochondrial metabolism known as reactive oxygen species, which damage DNA.)

To test their theory, Blau and Mourkioti treated affected animals with two different antioxidants to neutralize the reactive oxygen species—one provided in the animals' diet and another injected into the abdomen. In each of the two groups, the 10 treated animals exhibited improvements in heart function and life span when compared to 10 control animals.

"We began the treatment when the animals were 8 weeks old, before they had begun to develop cardiac symptoms of the disorder," Blau said. "But it may be that treatment even earlier would have an even more marked effect."

Although very encouraging, researchers caution that further studies are required to determine the effect of early antioxidant treatment on patients with Duchenne muscular dystrophy.

"The important thing is that we finally have a mouse model with which we can begin testing a number of potential therapies," Blau said. "Until now, no one really understood the cardiac basis of the disease, and clinicians have been prescribing nonspecific treatments. Now we can develop more specific drugs for patients that target the cause of their cardiac dysfunction."

The new mouse model may also be applicable to the study of other inherited conditions.

"Seeing this in the heart gave us new insight," said Blau. "It's possible that the effect of shortened telomeres may be relevant to diseases other than that caused by Duchenne muscular dystrophy. Many mouse models that now fail to recapitulate human diseases may be improved by similar shortening of telomeres."

More information: Role of telomere dysfunction in cardiac failure in Duchenne muscular dystrophy, DOI: 10.1038/ncb2790

Provided by Stanford University Medical Center