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## LARGE Protein Can Overcome Defects In Some Types Of Muscular Dystrophy

Muscular dystrophy is a group of genetic diseases characterized by progressive muscle degeneration. Working with mice with a type of the disease, researchers have found that by expressing an enzyme that attaches sugar molecules to a protein essential for proper muscle structure, they can restore normal muscle function.

Interestingly, the scientists found evidence of similar benefits when they expressed the protein, known as LARGE, in cells from patients with similar types of muscular dystrophies with distinct gene defects, suggesting that this approach may have clinical benefits for patients with the debilitating disease.

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— **Kevin P. Campbell**

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The study, led by Howard Hughes Medical Institute investigator Kevin P. Campbell at the University of Iowa College of Medicine, was published online in the journal *Nature Medicine* on June 6, 2004. Campbell's co-authors on the paper were from the University of Iowa, the University of Toronto, Uppsala University in Sweden, and the National Center of Neurology and Psychiatry in Tokyo. The study complements additional work by Campbell and colleagues from the Scripps Research Institute in California, the California Pacific Medical Center Research Institute, and Uppsala University, which elucidated the critical role of LARGE in the processing of a protein required to link muscle cells to their surrounding matrix. This work was published in an advance online publication of *Cell* on June 3, 2004.

A subset of muscular dystrophies have recently been linked to mutations in a group of enzymes involved in adding sugars to the muscle protein alpha-dystroglycan, in a process known as glycosylation. Without the attached sugars, alpha-dystroglycan is unable to carry out its function of

linking the internal structural proteins of muscle cells to the surrounding extracellular matrix, providing essential structural support that protects the muscle membrane from contraction-induced damage.

Intense muscle activity, particularly when combined with stretching, such as running downhill or walking down stairs, places stress on a muscle cell. In normal muscle, the link between alpha-dystroglycan and the surrounding matrix protects against this stress. According to Campbell, in patients with muscular dystrophy the absence of this link makes the muscle much more susceptible to damage.

“In most cases, if a normal person went out and ran 20 miles, the muscle is going to be damaged. But the muscle is actually able to repair itself,” said Campbell. “However, patients with muscular dystrophy undergo this process much more quickly, and they eventually lose the ability to repair. That’s when they get weak.”

Defects in enzymes that transfer sugar molecules, known as glycosyltransferases, have been implicated in at least six different types of muscular dystrophy, according to Campbell. Therefore, the researchers wondered whether restoring glycosyltransferase activity would correct the defects in the muscle. To test this idea, they used mice in which the gene for a particular glycosyltransferase known as LARGE is mutated.

Mice without functional LARGE have a type of muscular dystrophy similar to that seen in a subset of patients, and are commonly used as a model for the disease. In these animals, alpha-dystroglycan lacks its attached sugars and cannot bind to the extracellular matrix.

In work described in the *Cell* publication, Campbell and colleagues identified LARGE as the critical enzyme for initiation of alpha-dystroglycan processing. They found a LARGE recognition motif in alpha-dystroglycan, and subsequently showed that without this recognition, alpha-dystroglycan is not functional in muscle. They propose that the interaction between LARGE and alpha-dystroglycan is a key determinant to maintain healthy muscle.

To increase the levels of LARGE in the mice, the researchers engineered a virus expressing the LARGE gene. When they injected the virus directly into the muscle of mice that were a few days old, the muscle cells produced functional LARGE protein.

Once LARGE was expressed in the muscle cells of the mice, the group investigated the effect this had on muscle structure and function. Examining alpha-dystroglycan, they found that it had been glycosylated and was able to bind to the extracellular matrix, restoring the proper link between the muscle cell and its surroundings. When viewed under a microscope, the muscle lacked the features associated with dystrophic muscle and instead had the appearance of healthy tissue. Importantly, the researchers also found that expression of LARGE did not have any pathological effects on normal muscle tissue when injected into healthy mice.

The researchers next tested the mice to determine whether the physiological changes seen with LARGE expression conferred functional benefits. Mice with and without the transferred gene were exercised by running downhill on a treadmill, and the researchers found that the muscular dystrophy mice who had the LARGE gene had significantly less muscle damage.

With the encouraging results from the mouse experiments, the researchers moved on to test whether addition of LARGE would have similar effects in the cells of patients with muscular dystrophy. Cells from patients with three different types of muscular dystrophy - Fukuyama congenital muscular dystrophy, muscle-eye-brain disease, and Walker-Warburg syndrome - were treated with the virus carrying the LARGE gene.

Indeed, the addition of the LARGE gene had an effect in the human cells similar to that seen in mice. Prior to the treatment, alpha-dystroglycan in the cells had lacked the normal sugar groups, but glycosylation was restored when the LARGE gene was expressed.

“What's nice about this is that cells from patients, which we can grow up in the laboratory, actually showed that we can correct the defect in their cells with LARGE,” Campbell said. Furthermore, although each of the cell types had a mutation in a different enzyme, glycosylation was similarly increased in all three, suggesting that increasing LARGE levels could be helpful regardless of the type of glycosyltransferase mutated in patients' cells.

“At least for all the glycosylation-related muscular dystrophies, we think that LARGE may be able to restore the function of alpha-dystroglycan. This has potential for developing therapy for a group of muscular dystrophies. If we could come up with a drug that would stimulate LARGE activity, then we could possibly bypass the defect that's seen in these different forms of muscular dystrophy,” Campbell noted.

He added that although it might also be possible to utilize gene therapy to introduce the LARGE gene into patients similar to what was done in the mice, there are still many challenges to be overcome in the administration of gene therapy, and a pharmaceutical approach may be more appropriate.

Campbell noted that the researchers had observed that even in normal tissue, LARGE increased the binding of alpha-dystroglycan to the extracellular matrix. This suggests that LARGE may be able to improve the link between muscle cells and their surrounding matrix even in muscular dystrophies that are not caused by glycosylation defects.