Using CRISPR to find muscular dystrophy treatments

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CRISPR-Cas9 gene editing technology is best known for its potential role in correcting genetic diseases. But it can also be used as a tool to find genes that act as supporting players, making the disease better or worse. Such genes might make good targets for new treatments.

A new study led by Louis Kunkel, Ph.D., and research fellow Angela Lek, Ph.D. at Boston Children's Hospital used CRISPR-Cas9 to better understand facioscapulohumeral muscular dystrophy (FSHD) and explore potential treatments. FSHD causes muscle weakness in the face, shoulder blades, and upper arms, and currently has no treatment other than supportive care.

In FSHD, the gene DUX4, normally active mainly during fetal development, is inappropriately "turned on." This causes toxic DUX4 protein to be produced in muscle cells when it shouldn't be, leading to cell death and muscle weakness.

Kunkel, Lek, and colleagues wondered if other genes could be targeted to prevent or compensate for this problem. They decided to use CRISPR-Cas9 to systematically mutate every gene in the genome. Their goal: to find genes that, when knocked out, enable human muscle cells to survive even when the DUX4 protein is being made.

"We essentially utilized the CRISPR screen technique as a shortcut to illuminate 'druggable' pathways for FSHD," says Lek, the paper's first author.

Preventing muscle cells from dying

The CRISPR-Cas9 screening process yielded about a half-dozen strong "hits." Among them were several genes that play a role in the cellular response to low-oxygen conditions, or hypoxia. That, it turns out, is the main driver of cell death caused by DUX4. When the team exposed muscle cells to compounds known to inhibit this hypoxia response, the cells stayed alive.

"Our results show that knockout of key genes involved in hypoxia signaling can desensitize cells to toxicity from DUX4, and prevent them from dying," says Kunkel.

Going a step further, the team created muscle cell lines from actual patients with FSHD. When treated with the same compounds, these cells showed fewer of the known biomarkers of the disease.

Finally, the researchers created two live zebrafish models of FSHD. When they exposed the fish to compounds that inhibit hypoxia signaling, the fish showed improvements in muscle structure and function and more swimming activity.

Moving forward

Kunkel and Lek have filed a patent application covering their discoveries. Lek, now at the Yale School of Medicine, is moving the drug experiments into mouse models of FSHD, while Kunkel plans further zebrafish studies at Boston Children's.

"The most encouraging finding about this study is that we discovered that there are FDA-approved drugs that can overcome DUX4's toxic effect," says Lek. "We now have a collection of drugs to test and figure out which is most suitable for long-term dosing in patients with FSHD."

Kunkel believes the process used in this study could be applied to many

other diseases.

"Our approach could provide an accelerated path to understanding complex genetic diseases, discovering therapeutic targets, and testing potential treatments," he says.

More information: A. Lek el al., "Applying genome-wide CRISPR-Cas9 screens for therapeutic discovery in facioscapulohumeral muscular dystrophy," *Science Translational Medicine* (2020). stm.sciencemag.org/lookup/doi/ … scitranslmed.aay0271

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