NEWS

CRISPR screen identifies therapeutic target for Facioscapulohumeral Muscular Dystrophy (FSHD)

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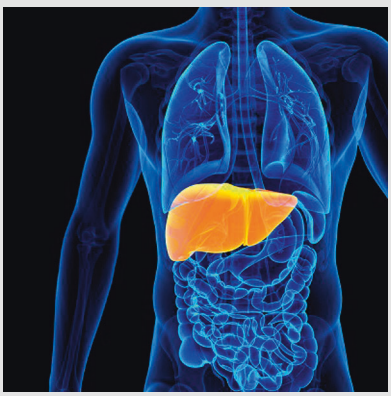
Difficulty in smiling fully, whistling, and throwing a ball are among the problems patients with facioscapulohumeral muscular dystrophy (FSHD) experience when symptoms begin, typically in adolescence. The muscle weakness and wasting disease, which worsens over time, most often affects the face, shoulder blades, and upper arms but can spread to other parts of the body. Although it is known that misexpression of the cytotoxic DUX4 protein is responsible for the disease, currently no treatment exists. In an article recently published in *Science Translational Medicine* (https://stm.sciencemag.org/content/12/536/eaay0271), Lek and colleagues report that hypoxia signaling is a key driver of and effective therapeutic target against DUX4-induced cell death. The researchers carried out a genome-wide CRISPR-Cas9 loss-of-function screen in an immortalized myoblast cell line known as MB135-DUX4i that harbors a doxycycline-inducible DUX4 transgene. Then they ran the most significant gene hits through GeneMANIA to investigate functional associations. This analysis identified hypoxia signaling as the most significant subnetwork of genes from the screen. Follow-up experiments revealed that high DUX4 expression led to stabilization and nuclear accumulation of HIF1A, a transcription factor and master regulator of hypoxia signaling, in the MB135-DUX4i cell line. HIF1A also colocalized with DUX4 in myotubes from a patient with FSHD. When the researchers knocked out HIF1A or other key hypoxia regulators, MB135-DUX4i cells became resistant to DUX4-induced toxicity and cell death. Next, the team treated MB135-DUX4i cells with several chemical inhibitors of hypoxia signaling to find that PI3K/Akt/mTOR inhibitors, including rapamycin, wortmannin, and herbimycin, effectively reduced DUX4-induced apoptosis. When Lek and colleagues treated patient-derived myogenic cells with the inhibitors, they saw the expression of three FSHD disease biomarkers drop to levels similar to that in healthy control cells. The finding demonstrated the therapeutic potential of hypoxia signaling inhibitors. Finally, the researchers conducted a short-term drug treatment in two DUX4 misexpression zebrafish models of FSHD. Fish treated with hypoxia signaling inhibitors displayed reduced apoptotic muscle fibers and swam more than untreated fish. When the researchers aged fish treated with herbimycin or rapamycin during development into adulthood, they found that the inhibitors prevented DUX4-induced muscle pathology in the adults. Altogether, the authors conclude that the results point to a targeted therapeutic approach for FSHD and provide a model for using genome-wide CRISPR-Cas9 screens to discover potential therapeutic targets for other rare genetic diseases. *—V. L. Dengler, News Editor*

Woman with rare genetic variant lowers potential therapy's risk profile



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Primary hyperoxaluria type 1 (PH1) is a rare autosomal recessive metabolic disorder characterized by recurring kidney and bladder stones. Often, the condition leads to early end-stage renal disease. Therapy to treat PH1 may require transplantation of both the liver and kidneys. Another potential approach, currently in phase I clinical trials, targets *HAO1*. *HAO1* encodes an enzyme known as hydroxyacid oxidase 1 that converts glycolate into glyoxylate, a substrate for the alanine-glyoxylate aminotransferase (AGXT). AGXT normally converts glyoxylate into glycine. However, when pathogenic variants render AGXT defective, glyoxylate instead converts to oxalate. PH1 results from overproduction of oxalate in the liver thanks to more than 175 variants in *AGXT*. Reducing HAO1 activity would decrease oxalate levels and increase glycolate, which is highly soluble and expected to be freely excreted. Although preclinical studies in rodents and monkeys did not demonstrate adverse effects, the risk of chronic suppression of *HAO1* is unknown. In a recent article published in the journal *eLife* (https://elifesciences.org/articles/54363?utm_source=content_alert&utm_medium=email&utm_content=fulltext&utm_campaign=25-March-20-elife-alert), McGregor and colleagues report that a healthy adult woman has been living with a complete germline knockout of *HAO1*. The researchers identified the woman via previous studies in which they had exome sequenced two large, population-based cohorts. The woman is homozygous for a frameshift variant, rs1186715161, that is predicted to truncate HAO1's C-terminus. The researchers reviewed her medical history and health records and conducted blood biochemical analyses. They found that her serum sodium, potassium, bicarbonate, chloride, creatinine, transaminases, and bilirubin were all normal, as were her plasma and urinary oxalate levels. As expected for *HAO1* loss of function, the woman's plasma and urinary glycolate levels were, respectively, 12 and 6 times greater than the upper limit for healthy reference individuals. The researchers estimate that the woman retains less than 2% hydroxyacid oxidase activity, yet she is a healthy adult and the mother of three healthy children. McGregor and colleagues conclude that these findings support the potential safety of HAO1 inhibition as a chronic therapy for PH1. Additionally, the team asserts that the reverse genetic approach taken in the study is a powerful means of de-risking potential drug targets. The authors propose that a collaborative, international database of human knockouts has the potential to accelerate therapeutic discovery and development for both rare and common diseases. *—V. L. Dengler, News Editor*

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