Researchers discover new factor in preventing phenylketonuria, offering new treatment strategy

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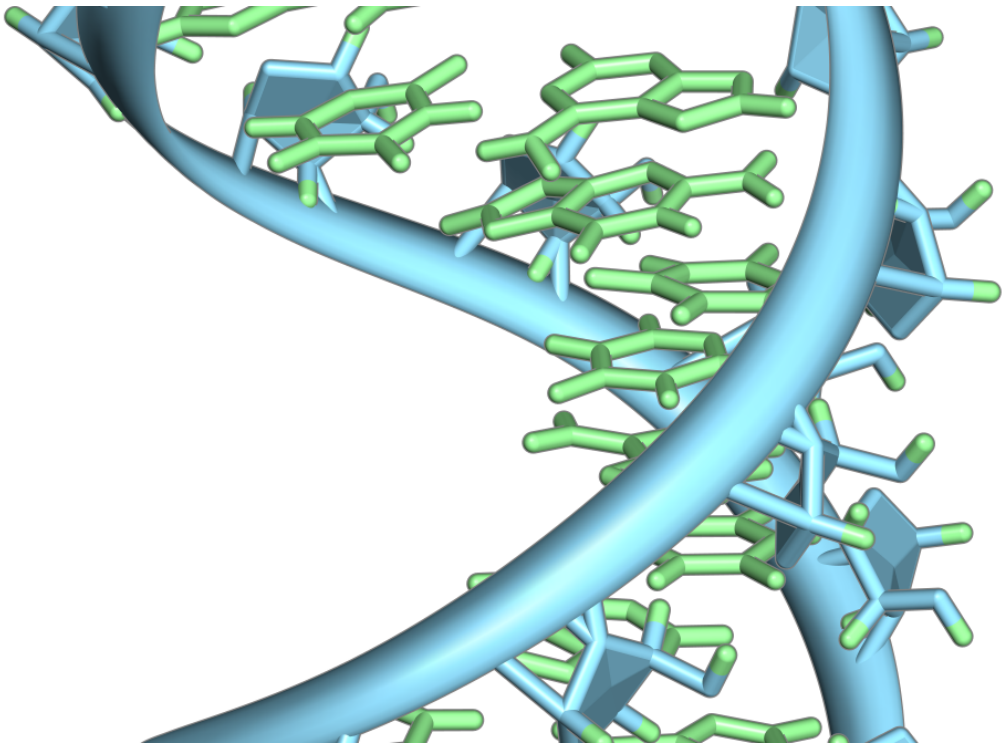

A hairpin loop from a pre-mRNA. Highlighted are the nucleobases (green) and the ribose-phosphate backbone (blue). Note that this is a single strand of RNA that folds back upon itself. Credit: Vossman/ Wikipedia

Researchers at The University of Texas MD Anderson Cancer Center have discovered a critical new factor in regulating phenylalanine

metabolism and, therefore, in preventing the inherited metabolic disorder phenylketonuria (PKU). The research also suggests a possible avenue for new treatments that may be effective for certain patients with PKU.

The study, published today in *Science*, demonstrates that the long noncoding RNA (lncRNA) HULC directly regulates the metabolic enzyme phenylalanine hydroxylase (PAH). Loss of the lncRNA resulted in excess phenylalanine and symptoms consist with PKU in model systems, whereas applying synthetic mimics of HULC restored PAH activity and lowered phenylalanine levels.

"There is a growing appreciation for the role of long noncoding RNAs in a variety of human diseases, but this is the first discovery of any long noncoding RNA involved with PKU," said co-senior author Liuqing Yang, Ph.D., associate professor of Molecular & Cellular Oncology. "Our research not only shows that HULC plays a very important role in PKU, but that we may be able to apply this discovery toward developing new treatments for patients who desperately need them."

Long noncoding RNAs are a form of RNA that do not encode a protein, but instead perform a variety of regulatory roles within the cell. Interested in identifying lncRNA with important biological functions, the researchers began this study by profiling lncRNAs expressed in various organs, both in early life and adulthood.

They discovered that HULC and its murine equivalent Pair were highly expressed in the adult liver. Hypothesizing this lncRNA may act as a tumor suppressor, the researchers genetically deleted Pair in mouse models. However, rather than developing liver cancer, the Pair knockouts instead developed metabolic symptoms consistent with PKU.

Phenylketonuria and its milder variant hyperphenylalaninemia (HPA)

are inherited metabolic disorders marked by an inability to convert the amino acid phenylalanine into tyrosine. These conditions affect roughly 1 in every 10,000 infants, most of whom have mutations in the PAH gene. Untreated PKU can lead to brain damage, intellectual disabilities, seizures and behavioral or psychiatric disorders. However, the only treatments available are a protein-restricted diet and/or supplementation with BH_4—a PAH cofactor.

Upon further examination, the researchers confirmed that Pair knockouts had excessive levels of phenylalanine. Biochemical studies demonstrated that both Pair and HULC bind directly to the PAH enzyme, enhancing its ability to convert phenylalanine into tyrosine.

"Phenylalanine hydroxylase has long been known to be regulated by allosteric factors, but we didn't know what those factors were until now. This represents the first identification of a non-substrate factor regulating PAH," said co-senior author Chunru Lin, M.D., Ph.D., associate professor of Molecular & Cellular Oncology. "Synthetic mimics of HULC were able to partially restore enzymatic activity in 13 of 17 different PAH mutants in vitro, representing some of the most frequent mutations seen in PKU."

The lncRNA mimics used in the study are synthetic nucleotides designed to be more stable and home to the liver for targeting PAH. To further investigate this approach as a possible treatment strategy, the researchers created a mouse model of PKU, carrying the most commonly found mutation in PAH. The HULC mimic improved phenylalanine metabolism as evidenced by a sustained reduction in phenylalanine levels.

Based on these results, the researchers anticipate that HULC mimics may be a useful strategy in treating patients with mutations in both the lncRNA as well as the PAH enzyme. In collaboration with experts at the

University Hospital of Nancy Reference Center for Inborn Errors of Metabolism (Nancy, France), the team is working to determine the prevalence of HULC mutations in patients with PKU as a possible alternate cause of the disease.

The team also is pursuing additional laboratory studies to advance HULC mimics toward future clinical studies to evaluate safety and efficacy.

The study was led by Yajuan Li, Ph.D., and Yaohua Zhang, Ph.D., both postdoctoral fellows in Molecular & Cellular Oncology, and Zhi Tan, M.D., Ph.D., now at the Baylor College of Medicine.

More information: A noncoding RNA modulator potentiates phenylalanine metabolism in mice, *Science* (2021). DOI: 10.1126/science.aba4991 , science.sciencemag.org/content/373/6555/662

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