Plug-and-play protein modification using Homology-independent Universal Genome Engineering (HiUGE)

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Video Abstract

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Abstract

The ability to label and manipulate proteins in the body is essential to modern biological research. Unfortunately, current methods, such as tagging with antibodies, are often inefficient and expensive. Even worse, researchers are realizing that many of the antibodies available just simply don't work. Now, a new molecular tool could help researchers break through that barrier. Researchers in the Soderling Laboratory of the Cell Biology Department at Duke University, have developed a high-throughput system capable of modifying entire panels of proteins using a new dual-vector gene-editing approach. Dubbed Homology-independent Universal Genome Engineering, this system allows for the dynamic visualization and functional manipulation of proteins both in vitro and in vivo, including in neurons. This is HiUGE. HiUGE isn't the first protein-modifying system to rely on gene editing. Techniques such as single-cell labeling of endogenous proteins (SLENDR) or homology-independent targeted integration (HITI) have made it possible to insert foreign DNA sequences into genes of interest. The difference: these methods require customized gene-specific donor vectors for each insertion; HiUGE doesn't. In HiUGE, the donor vector contains an insertional DNA payload flanked by an artificial DNA sequence non-homologous to the target genome. This sequence is recognized by a donor-specific guide RNA that autonomously directs Cas9-mediated payload clipping and release. Separate, gene-specific gRNA vectors then designate the payload target in the gene of interest. This design frees the donor vectors of any gene-specific sequences. The result is the potential to create high-throughput donor "toolkits" that target a variety of genes rather than just one. In addition, HiUGE employs adeno-associated virus as an efficient vehicle to deliver these "toolkits" to cells or even tissues in animals. As a proof of concept, the research team co-transduced primary neurons from neonatal mouse pups with two sets of vectors: one containing gRNA targeting the mouse *Tubb3* gene and the other containing the machinery to insert the protein tag hemagglutinin, or HA. After about one week, fluorescence detection revealed successful HA labeling. Genomic insertion of the payload was verified by sequencing the *Tubb3* locus. In further tests, HiUGE proved capable of targeting multiple genomic loci for protein labeling, labeling proteins in vivo, delivering different payloads interchangeably at a single genomic locus, and targeting specific neural circuits. Potential drawbacks of all CRISPR-dependent systems, including HiUGE, is the formation of indels at the targeted loci or off-target insertion of genomic payloads. The team found with careful design these effects can be greatly minimized. And the benefits are very promising. Scalable, efficient, and universally compatible for virtually any loci accessible by CRISPR/Cas9, HiUGE opens the door to pairing high-throughput "omics" with experimental validation and phenotypic screening to address molecular mechanisms of cellular biology.