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RESEARCHERS AT BROAD INSTITUTE AND MIT ENGINEER "CAS9" ANIMAL MODELS TO STUDY DISEASE AND INFORM DRUG DISCOVERY

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Researchers from the Broad Institute and Massachusetts Institute of Technology have created a new mouse model to simplify application of the CRISPR-Cas9 system for *in vivo* genome editing experiments. The researchers successfully used the new "Cas9 mouse" model to edit multiple genes in a variety of cell types, and to model lung adenocarcinoma, one of the most lethal human cancers. The mouse has already been made available to the scientific community and is being used by researchers at more than a dozen institutions. A paper describing this new model (http://www.cell.com/cell/abstract/S0092-8674(14)01163-5) and its initial applications in oncology appears this week in *Cell*.

In recent years, genetic studies have found thousands of links between genes and various diseases. But in order to prove that a specific gene is playing a role in the development of the disease, researchers need a way to perturb it – that is, turn the gene off, turn it on, or otherwise alter it – and study the effects.

The CRISPR-Cas9 genome-editing system is one of the most convenient methods available for making these alterations in the genome. While the tool is already being used to test the effects of mutations *in vitro* – in cultured cell lines, for instance – it is now possible to use this tool to study gene functions using intact biological systems.

The CRISPR-Cas9 system relies on two key features to edit the genome: Cas9, a "cleaving" enzyme capable of cutting DNA; and guide RNA, a sequence that directs Cas9 to the DNA target of interest in the genome. However, the Cas9 enzyme presents some delivery challenges for *in vivo* applications.

"By equipping the mouse with Cas9, we relieved the burden of delivery. This frees up space for the delivery of additional elements – whether by viruses or nanoparticles – making it possible to simultaneously mutate multiple genes and even make precise changes in DNA sequences," said Randall Platt, a graduate student at MIT working at the Broad Institute in the lab of Feng Zhang, an assistant professor at the McGovern Institute for Brain Research at MIT, and a core member of the Broad Institute. Platt and Sidi Chen, a postdoctoral fellow at MIT's Koch Institute for Integrative Cancer Research working in the lab of Institute Professor Phillip Sharp, were co-first authors of the paper.

This ability to perturb multiple genes at the same time may be particularly useful in studying complex diseases, such as cancer, where mutations in more than one gene may be driving the disease. To demonstrate a potential application for cancer research, the authors used the "Cas9 mouse" to model lung adenocarcinoma. Previously, scientists working with animal models have had to knock out one gene at a time, or cross animal models to produce one with the needed genetic modifications, processes that are challenging and time consuming.

"The 'Cas9 mouse' allows researchers to more easily perturb multiple genes *in vivo*," said Zhang, who, along with Sharp, served as co-senior author of the *Cell* paper. "The goal in developing the mouse was to empower researchers so that they can more rapidly screen through the long list of genes that have been implicated in disease and normal biological processes."

Researchers contributing to the paper also found that cells derived from the "Cas9 mouse" could be extracted for use in lab experiments and were able to leverage the Cas9-expressing cells to edit immune dendritic cells even after the cells had been removed from the mouse, allowing the researchers to experiment with cells that aren't easily accessible and often lack the shelf life to conduct such experiments.

"As we demonstrated with immune cells, the mouse allows us to experiment with cells that only remain viable for a few days *ex vivo* by leveraging the fact that they already express Cas9. Absent the expression of Cas9, we would not have sufficient time for the CRISPR system to work its magic," said Broad core member and paper co-author Aviv Regev, who is an associate professor of biology at MIT. Regev's lab, along with the lab of Broad senior associate member Nir Hacohen (a faculty member at Massachusetts General Hospital and Harvard Medical School), used the mouse to investigate dendritic cells, as reported in the *Cell* study.

"Genetic manipulation is one of the most critical tools we have for investigating complex circuits, and the 'Cas9 mouse' will help us do it more effectively," said Regev.

The "Cas9 mouse" has been deposited with Jackson Laboratory, where it is available to the entire scientific community by request.

The study was supported by the National Science Foundation; The Damon Runyon Cancer Research Institute; Simons Center for the Social Brain at MIT; the National Institute of Health's National Human Genome Research Institute, National Cancer Institute, and National Institute of Mental Health; Helmsley Charitable Trust; Klarman Cell Observatory; the National Cancer Institute; the Koch Institute for Integrative Cancer Research; the Broad Institute's Stanley Center for Psychiatric Research; Howard Hughes Medical Institute; the Marie D. and Pierre Casimir-Lambert Fund; Bob Metcalfe; and the Keck, Searle Scholars, Klingenstein, Vallee, and Merkin Foundations.

Other researchers who worked on the study include: Yang Zhou, Michael Yim, Lukasz Swiech, Hannah Kempton, James Dahlman, Oren Parnas, Thomas Eisenhaure, Marko Jovanovico, Daniel Graham, Siddharth Jhunjhunwala, Ramnik Xavier, Robert Langer, Daniel Anderson, and Guoping Feng.

About the engineered CRISPR-Cas9 system

CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) have recently been harnessed as genome editing tools in a wide range of species. The engineered CRISPR-Cas9 system allows researchers to mutate or change the expression of genes in living cells, including those of humans. The family of Cas9 nucleases (also known as Cas5, Csn1, or Csx12) recognizes DNA targets in complex with RNA guides. Researchers can now harness the engineered system to home in on specific nucleic acid sequences and cut the DNA at those precise targets. The cuts modify the activity of the targeted genes, allowing researchers to study the genes' function.

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to empower this generation of creative scientists to transform medicine. The Broad Institute seeks to describe all the molecular components of life and their connections; discover the molecular basis of major human diseases; develop effective new approaches to diagnostics and therapeutics; and disseminate discoveries, tools, methods and data openly to the entire scientific community.

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Paper(s) cited:

Platt, R. *et al.* "CRISPR-Cas9 knockin mice for genome editing and cancer modeling (http://www.cell.com/cell/abstract/S0092-8674(14)01163-5)." *Cell*. September 25, 2014. DOI: 10.1016/j.cell.2014.09.014