CRISPR/Cas9 used for rapid functional study of cancer-causing genes

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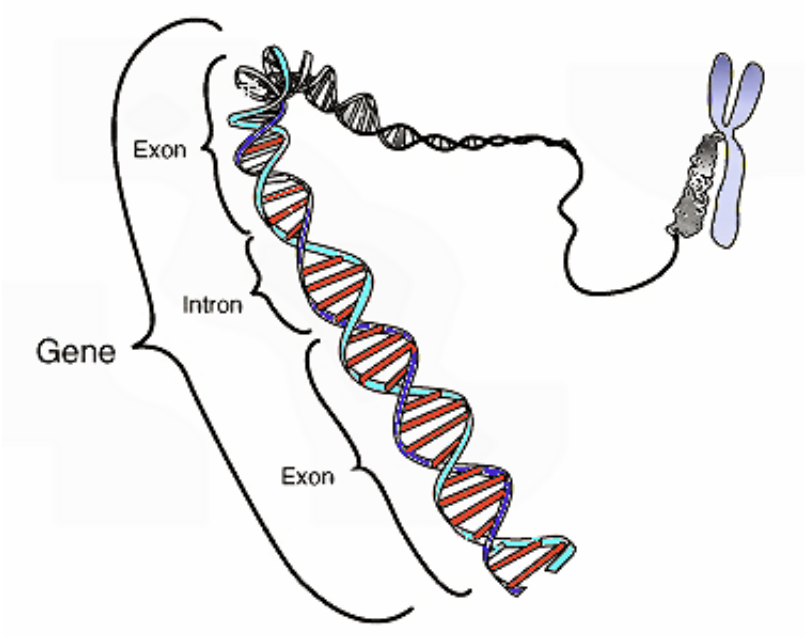

This image shows the coding region in a segment of eukaryotic DNA. Credit: National Human Genome Research Institute

In a novel use of the CRISPR/Cas9 system, which can be deployed to switch genes off, researchers from Germany, the UK and Spain have developed a multiplexed screening approach to study and model cancer development in mice. The scientists mutated genes in the adult mouse liver uncovering their cancer-causing roles and determining which combinations of genes cooperate to cause liver cancer.

The approach is rapid, highly flexible, efficient and scalable, allowing the team to study many genes simultaneously or to examine large regions of the genome. By engineering mutations directly in adult mice and thus bypassing the need to create transgenic animals, the method can more rapidly deliver results and, in some instances, more closely mimic the biological processes operating in human cancers.

Cancer is a disease of DNA, in which hundreds or thousands of mutations develop in a cancer cell, confounding DNA-sequencing efforts to distinguish the cancer-driving mutations from the 'passenger' mutations that are side-effects of cancer development. In addition, many cancer genes are difficult to discover by sequencing, for example, because they are not mutated, but dysregulated by other means in cancer. Another major challenge is to determine the function of mutated genes in normal cells and understand how their mutation causes cancer.

CRISPR/Cas9 is a method that enables researchers to target mutations to any position in the genome, switching off one gene or several genes. The application of this technology in model organisms is however still in its infancy. In this study, the team used CRISPR/Cas9 to cause mutations in liver cells of adult mice in order to trigger liver cancer development. With approximately 800,000 new cases per year, liver cancer is the sixth most-prevalent cancer in the world.

"We reasoned that, by targeting mutations directly to adult liver cells using CRISPR/Cas9, we could better study and understand the biology of this important cancer," says, Dr Mathias Friedrich, an author on the paper from the Wellcome Trust Sanger Institute. "Other approaches to engineer mutations in mice, such as stem cell manipulation, are limited by the laborious process, the long time frames and large numbers of animals needed. And, our method better mimics important aspects of human cancer biology than many "classic" mouse models: as in most human cancers, the mutations occur in the adult and only affect few

cells".

The team of researchers developed a list of up to eighteen genes with known or unknown evidence for their importance in two forms of liver cancer. They introduced CRISPR/Cas9 molecules targeting various combinations of these genes into mice. The mice developed liver or bile duct cancer within a few months.

"Our approach enables us to simultaneously target multiple putative genes in individual cells", says Professor Roland Rad, project leader at the Technical University of Munich and the German Cancer Research Center Heidelberg. "We can now rapidly and efficiently screen which genes are cancer-causing and which ones are not. And, we can study how genes work together to cause cancers—a crucial piece of the puzzle we must solve to understand and tackle the disease."

This approach confirmed that a set of proteins called ARID, which influence the organisation of chromosomes, are important for liver cancer development. Mutations in a second protein, TET2, were found to be causative in bile duct cancer: although TET2 has not been found to be mutated in human biliary cancers, the proteins that it interacts with have been, showing that the CRISPR/Cas9 method can identify human cancer genes that are not mutated, but whose function is disturbed by other events.

"The new tools of targeting genes in combination and inducing insertions or deletions in chromosomes change our ability to identify new cancer-causing genes and to understand their role in cancer," says Professor Allan Bradley, Senior group leader and Director Emeritus from the Sanger Institute . "Our results show that this approach is feasible and productive in liver cancer; we will now continue to study our new findings and try to extend the approach to other cancer types."

The team's CRISPR/Cas9 approach is also promising for the biological exploitation of genomic deserts—regions of the human genome that appear to be bereft of genes. Recent studies, such as the ENCODE Project, suggest that deserts can be populated, if not by genes, then by regulatory DNA regions that influence the activity of genes.

"Liver cancer has many DNA alterations in regions lacking genes: we don't know which of these might be important for the disease," explains Roland Rad. "We could show that it is now possible to delete such regions to systematically determine their role in liver cancer development. "

The team's new approach will allow them to study the importance of other deserts by removing them using CRISPR/Cas9 and studying the consequences for cell, tissue and animal biology.

More information: Weber J, Öllinger R et al. (2015) CRISPR/Cas9 somatic multiplex-mutagenesis for high-throughput functional cancer genomics in mice. *Proceedings of the National Academy of Sciences* (2015) www.pnas.org/cgi/doi/10.1073/pnas.1512392112

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