Researchers create first targeted knockout rats using zinc finger nuclease technology

July 23 2009

Scientists from The Medical College of Wisconsin in Milwaukee, Sangamo Biosciences, Inc., Sigma-Aldrich Corporation, Open Monoclonal Technology, Inc. (OMT) and INSERM today announced the creation of the first genetically modified mammals developed using zinc finger nuclease (ZFN) technology.

In a paper published in the July 24, 2009 issue of *Science*, researchers describe the novel application of ZFNs to generate rats with permanent, heritable gene mutations, paving the way for the development of novel genetically modified animal models of human disease. ZFN technology will make the generation of such animals faster and will create new opportunities in species other than mice.

"Until now, rat geneticists lacked a viable technique for "knocking out," or mutating, specific genes to understand their function," said Howard Jacob, Ph.D. Director of the Human and Molecular Genetics Center at the Medical College of Wisconsin. "This study demonstrates that ZFN technology bypasses the current need to conduct cumbersome experiments involving nuclear transfer (cloning) or embryonic stem cells and allows rapid creation of new animal models."

In the study published in *Science* titled "Knockout Rats via Embryo Microinjection of Zinc Finger Nucleases," (Geurts, et al.) scientists used ZFNs to knock out an inserted reporter gene and two native rat genes without causing measurable effects on other genes. Importantly, offspring of the ZFN-mutated rats also carried the modifications,

demonstrating the genetic changes were permanent and heritable. Together, these results demonstrate the ability to deliver engineered ZFNs into early-stage embryos and rapidly generate heritable, knockout mutations in a whole organism.

Rats are physiologically more similar to humans than are mice for many traits and are ideal subjects for modeling human diseases. Extensive genetic characterization has revealed that approximately 90 percent of the rat's 25,000-30,000 estimated genes are analogous to those in humans and mice, and their larger size makes them a superior model for drug-evaluation studies using serial sampling. Generating rats with knockout mutations has been a major challenge, but the new technique will increase the rat's usefulness in research pertaining to physiology, endocrinology, neurology, metabolism, parasitology, growth and development and cancer. Along with his colleagues, Dr. Jacob's team hopes to use knockout rats to gain a better understanding of disease processes related to hypertension, heart disease, kidney failure and cancer.

ZFNs are engineered proteins that induce double strand breaks at specific sites in an organism's DNA. Such double-strand breaks stimulate the cell's natural DNA-repair pathways and can result in site-specific changes in the DNA sequence. Previously, ZFNs were used to knock out specific genes in fruit flies, worms, cultured human cells and zebrafish embryos and are now in human clinical trials for the treatment of HIV/AIDS. This is the first example of successful gene editing in mammalian embryos using this technology.

"Our ZFN technology is widely applicable across species," stated Philip Gregory, D.Phil., Sangamo's vice president of research. "Used in conjunction with standard laboratory techniques, ZFNs provide a powerful solution to the challenge of making gene knockouts in cells and in whole organisms. We believe that this technology will become the

method of choice for genome engineering in cells, plants and transgenic animals."

In the first commercial application of this technique, OMT, a private biotechnology company developing a new rat-based human antibody platform, used Sangamo's ZFNs to knock out the gene encoding rat immunoglobulin M (IgM), an important gene for rat antibody production. Inactivation of rat IgM expression is the first step in generating rats that exclusively express human antibodies encoded by transgenic human immunoglobulin genes. "Creating a knockout rat was the biggest challenge OMT faced", said Dr. Roland Buelow, CEO of OMT and a senior author of the paper. "Inactivation of endogenous rat antibody expression is essential for human antibody expression in genetically engineered animals. To solve this problem, we used ZFN technology in an application that has the potential to revolutionize genetic engineering of animals."

"We have invested our time and resources to develop the CompoZr platform because we see enormous potential in a technology that can precisely manipulate the genome of living organisms," said Dr. David Smoller, President of Sigma-Aldrich's Research Biotech business unit. "We are proud to be part of the public-private collaboration developing the proof-of-concept for this technique, which we believe will become the standard for the creation of genetically engineered research animals."

Source: Medical College of Wisconsin (news : web)