Laboratory Animal Research Center

Division of Animal Genetics
先進動物ゲノム研究分野

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Genome engineering technologies achieve a "revolution" in life science and medical science. These techniques allow us to manipulate genes of interest for several purposes. Using those technologies, we have developed many useful strains in mice and rats. We are now focusing on generating "humanized animals" or "immunodeficient animals". These valuable animals can be used for xenotransplantation of human cells/tissues including human HSCs and IPSCs. We are also developing therapeutic strategies such as cell therapy and gene therapy with genome editing tools.

Characterization of several severe combined immunodeficiency rats for humanized models

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The immunodeficiency animals are valuable experimental models, not only in the studies of immunodeficiency related diseases, they also have good performances in the application of grafting various tissues. Therefore, the immunodeficiency animals have been widely applied in generation of humanize animals, regeneration medical, tumor researches, etc. By utilizing the CRISPR/Cas9 genome editing tool, we generated a Severe Combined Immunodeficiency (SCID) rat model, which carry homozygous mutation in both Il2rg and Rag2 gene. These combined mutations caused the retard of both T cell and B cell development, as well as the deficiency of functional NK cells and cytokines secretion, providing favorable in vivo environment for the subsistence and proliferation of exogenous cells or tissues. Other than the immunodeficiency animals that generated by combining the mutations from different rat strains, our SCID rats have a clear genetic background of F344 rats. Our SCID rats has been set up a Bio-recourse project, and provided to institutes and researchers all over the world. In the following studies, we devote to modifying other genes in these SCID rats, to improve the efficiency of xenograft and alleviate acute xenogeneic graft-versus-host disease (GVHD) in the recipient SCID rats.

Efficient and precise gene disruption with CRISPR-Cas3 in human T cells

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Chimeric Antigen Receptor (CAR)-T cell therapy is promising cancer immunotherapy. Conventionally, CAR-T cells are produced from autologous T cells, but this can be high costs, long manufacturing periods, and difficulties in ensuring uniform quality of cell sources. We reported that genome editing using CRISPR-Cas3 system is possible in human cells. The novel genomic editing system can produce fewer off-target and mosaic mutations compared to CRISPR-Cas9, which is the most widely used genome editing tools. Our research aims are to use CRISPR-Cas3 to generate a safe and effective CAR-T therapy. To overcome the limitations of producing autologous CAR-T cells, we investigated whether CRISPR-Cas3 system induces genetic modifications on genes involved in graft-versus-host disease and immune rejection in Jurkat cells, a human acute T cell leukemia cell line. As a result of this experiment, it caused loss of function of the target genes and we found that there were no off-target mutations observed in its cells. In addition, this system can also generate targeted deletions of the target genes in human primary T cells. These results suggest that the CRISPR-Cas3 system could be a powerful genetic tool for generating allogenic CAR-T cells.

Generation of several genetically engineered mice and rat models via genome editing technologies

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CRISPR-Cas9 systems have been widely used for gene targeting in mice and rats. The non-homologous end joining (NHEJ) repair pathway, which is dominant in zygotes, efficiently induces insertion or deletion (indel) mutations as gene knockouts (KOs) at targeted sites, whereas gene knock-ins (KIs) via homology-directed repair (HDR) are difficult to generate.

We have developed the two KI strategies with CRISPR/Cas9 for the large genomic regions in rodents. One is the long single strand DNA (lssDNA)-mediated KI method. Microinjection and electroporation of originally synthesized lssDNAs with gRNA and Cas9 mRNA could produce several types of KI mice and rats with a good efficiency such as GFP-tagging, floxed and repeat sequence replacement. In addition, we have also used a double-stranded DNA (dsDNA) donor template with Cas9 and two single guide RNAs (sgRNAs), one designed to cut the targeted genome sequences and the other to cut both the flanked genomic region and one homology arm of the dsDNA plasmid, resulting in 20%–50% KI efficiency among G0 pups. This combinational method of NHEJ and HDR mediated by the CRISPR-Cas9 system, named Combi-CRISPR, facilitates the efficient and precise KIs of plasmid DNA cassettes in mice and rats.

We have established genetically-modified mice and rats via these genome editing strategies with several collaborators.

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