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 have advanced life science and medical research through precise genetic manipulation. We have generated diverse mouse and rat models, particularly humanized and immunodeficient animals for xenotransplantation studies. In parallel, we are developing safe and effective genome editing strategies based on the CRISPR-Cas3 system, which offers high target specificity and large-deletion capability. These technologies are applied to gene therapy, cell engineering, and disease modeling, while national platform projects support rat research through resource development and distribution.

CRISPR-Cas3 mediated gene therapy for Transthyretin Amyloidosis

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Gene therapy using genome editing holds promise as a fundamental treatment for hereditary diseases, and its efficacy has been confirmed in several recent clinical trials. However, concerns regarding safety remain. We have been developing a CRISPR/Cas3 system, which has a longer target recognition sequence compared to Cas9, thereby reducing the risk of off-target effects. Additionally, Cas3 exhibits the unique capability to induce large deletions in the genome, making it a promising tool for achieving precise and safe genetic modifications. Our research focuses on developing safer in vivo gene therapy approaches using the CRISPR/Cas3 system.

Transthyretin amyloidosis (ATTR) is a systemic disorder caused by the deposition of misfolded transthyretin (TTR) proteins in various organs, leading to organ dysfunction. ATTR can be classified into two types: hereditary ATTR (ATTRv), which is caused by mutations in the TTR gene, and wild-type ATTR (ATTRwt), which develops with aging in the absence of TTR mutations. Current treatments primarily include siRNA-based therapies to suppress TTR production and TTR stabilizers to prevent fibril formation; however, these treatments require continuous administration. Therefore, the development of genome-editing-based therapies offers a promising alternative for a more permanent solution.

In our study, we successfully achieved approximately 75% reduction in serum TTR levels — a threshold associated with clinical efficacy — by delivering modified mRNA encoding CRISPR/Cas3 via lipid nanoparticles to the liver, which produces about 90% of TTR. Unlike CRISPR/Cas9, which has been reported to introduce new mutations and off-target effects, our CRISPR/Cas3-based approach did not result in the generation of mutated TTR variants, demonstrating its superior safety profile.

This study provides a novel genome-editing strategy using CRISPR/Cas3, offering a safer and more effective therapeutic option for the treatment of various hereditary diseases, including ATTR.

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

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The CRISPR-Cas9 system is widely used as genome editing tools all over the world. Its high efficiency of genome editing leads to development of a lot of novel therapeutic modalities; however, off-target genome editing should be carefully evaluated in terms of safety issues caused by unintended and undesirable mutations in human genome. The CRISPR-Cas3 system has been shown to have lower off-target activity compared to Cas9. Here, we demonstrated successful application of the CRISPR-Cas3 system to generate allogenic T cells by deleting two clinically relevant genes, the endogenous T-cell receptor (*TRAC*) and beta-2 microglobulin (*B2M*), in human T cells to reduce activity of graft-versus-host defence and immune rejection. Importantly, no off-target mutations were observed in *TRAC* or *B2M* deleted cells generated with the CRISPR-Cas3 system, whereas off-target mutations existed with the CRISPR-Cas9. Thus, the CRISPR-Cas3 system is advantageous strategy in terms of safety concerns for development of allogenic T cell therapies.

Resource and Model Animal Production Support - NBRP-Rat and AdAMS to promote Rat Research

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The rat has a history of more than 100 years as a laboratory animal, accumulating physiological and pharmacological data. As the development of genome editing technology progresses, modifying genes in rats has become much easier, making laboratory rats more valuable in contemporary research. Various genome editing technologies have been developed and utilized to create useful rat models.

Participation in two platform projects has actively promoted rat research. The National Bio Resource Project–Rat (NBRP-Rat), launched in 2002 with Kyoto University as the core institution, is a world-class resource center that has collected and preserved more than 800 rat strains to date. Under genetically and microbiologically controlled conditions, three immunodeficient rat strains—Il2rg knockout, Rag2 knockout, and Il2rg/Rag2 double knockout—are provided to researchers. To date, more than 150 material transfer agreements (MTAs) have been concluded, resulting in the distribution of over 1,000 immunodeficient rats.

As a member of the Advanced Animal Model Support Platform, we provide support for the generation of genetically engineered rats. To date, more than 120 rat strains have been successfully generated, including strains developed in response to requests from domestic researchers to support life science research.

Publications

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