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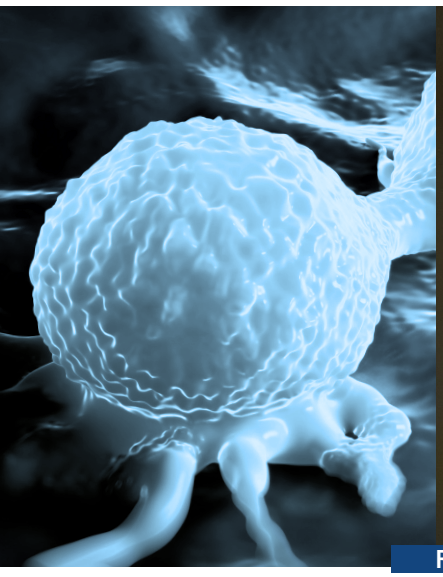

RESEARCH MODELS

NOD CRISPR *Prkdc Il2r gamma* (NCG) Triple-Immunodeficient Mouse

Nomenclature:

NOD-*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}/NjuCrl

Distinguishing Features:

- Deletion of *Prkdc* and *Il2rg* genes via CRISPR-Cas9
- Deficient in mature T, B, and functional NK cells
- Engraftment of human tissue, including primary tumor cells
- No license required for academic or commercial purposes

History of Immunodeficient Mice

Standard immunodeficient mice have been used for several decades, with the intent to study and transplant foreign tissue. The most commonly used are either "nude" (hairless) mice that lack mature T cells, or severe combined immunodeficiency (SCID) mice that lack both functional T and B cells.[1,2] Despite displaying several defects in immunity, these mice had limitations in engrafting foreign tissue, including with human immune cells when attempting to generate a "humanized" immune system, due to their partially functional immune system.[3,4]

Triple-immunodeficient mice address these issues, lacking functional/mature T, B, and NK cells, along with reduced macrophage and dendritic cell function. This is accomplished by combining mutations from three different genes: the *Sirpa* mutation, found in the NOD mouse; and the *Il2rg* and *Prkdc*, part of the SCID family of genes. The NOD/Nju carries a mutation in the *Sirpa* gene that increases efficiency of engrafting foreign hematopoietic stem cells. The *Prkdc* gene is required for V(D)J recombination in T- and B-cell maturation, while the *Il2rg* gene encodes for the common interleukin-2 receptor gamma subunit required for cytokine signaling of several interleukin receptors.[5] The loss of cytokine signaling reduces the function and maturation of T, B, NK, and dendritic cells, compounding the effects of the *Prkdc* mutation. The perturbation of these three critical genes results in a triple-immunodeficient mouse that can be used for applications requiring significantly impaired immune function, including engraftment of primary tumors from human patients.

The First CRISPR Immunodeficient Model

Uniquely created using CRISPR-Cas9 technology to alter to the *Prkdc* and *Il2rg* genes, the NCG mouse is the first CRISPR-generated immunodeficient model Charles River has to offer. The NCG is similar to other triple-immunodeficient models in that it is capable of hosting xenograft cells, tissue, and human immune system components, thus enabling researchers to further study tumor biology and immuno-oncology, hematopoiesis, infectious disease, GvHD, and human organ development and function.

Research Applications

- Oncology
- Immunology
- Regenerative medicine
- Infectious disease

Origin and Background

The NCG nomenclature is NOD-*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}/NjuCrl and was co-developed by Nanjing Biomedical Research Institute of Nanjing University and Nanjing Galaxy Biopharma in 2014, and transferred to Charles River in 2016. This model was created by sequential CRISPR editing of the *Prkdc* and *Il2rg* loci in the NOD/Nju mouse, generating a mouse coisogenic to the NOD/Nju. The NOD/Nju carries a mutation in the *Sirpa* gene that increases efficiency for engrafting of foreign hematopoietic stem cells. Additionally, the NOD background displays reduced NK and macrophage cell function. The *Prkdc* gene was knocked out by a 52-base-pair deletion in exon 37, generating a SCID-like phenotype lacking proper T cell and B cell maturation. The *Il2rg* gene was knocked out by a 22-base-pair deletion in exon 3, exacerbating the SCID phenotype by further impairing T and B cell growth and maturation while causing a decrease in NK cell function. Cas9 mRNA and guide RNA were co-injected into Nod/Nju zygotes.[6] Cas9 endonucleases then generated a DNA double-strand break (DSB) at the targeted genome locus, subsequently repaired through error-prone nonhomologous end joining (NHEJ). In the absence of a template, NHEJ is activated, resulting in insertions and/or deletions (indels) that disrupt the target loci. NCG mice are homozygous for the *Prkdc* deletion, while, for the *Il2rg* deletion, female mice are homozygous and males hemizygous, as the *Il2rg* gene is carried on the X chromosome.

Applications

- Immunology applications, including the study of human immune system responses or hematopoiesis in the development of human immune cells
- Oncology applications for the engraftment of primary human tumor cells, either solid or liquid, as well as patient-derived xenografts (PDX)
- Infectious disease applications analyzing human immune cell responses to infectious agents and viruses such as HIV, Epstein-Barr and dengue
- Diabetes applications using double-engrafted mice assessing the human immune system response to human pancreatic islets
- Regenerative medicine applications, including human organ transplantation and tissue regeneration using human stem cells

References

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