MOUSE GENOME EDITING CORE UNIT

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OVERVIEW

The term "cancer" encompasses a whole spectrum of extremely complex diseases. Genetic and epigenetic modifications in tumour cells lead to the acquisition of a "malignant" phenotype that enables them to escape normal physiological control. We can accurately reproduce many of these modifications in the mouse, creating animal models to study the disease. Tumour cells also interact, at different levels, with other cells in the body such as those of the tumour stroma, immune, cardiovascular or lymphatic systems, which, in turn, modulate tumour growth, invasion and expansion. The study of such complexity requires *in vivo* models that reproduce all the features of cancer in a "whole body" context, including the specific genetic alterations that lead to tumour development in each particular tumour. The precise, targeted and controlled modification of the mouse genome, using the most advanced genome editing tools, sustains the generation of genetic mouse models of cancer that are crucial for understanding the molecular basis of tumour development and the preclinical validation of new and more efficient cancer therapies.

"The Mouse Genome Editing Unit has more than 20 years of experience in the design, generation, and validation of genetically modified mouse models using state-of-the-art genome editing techniques."

RESEARCH HIGHLIGHTS

COVID19 preclinical mouse models

One limitation in COVID research is the lack of adequate models to study SARS-CoV-2 infection, especially animal models, where the complex interactions established between the virus and its host can be reproduced in a physiological context. During 2021, the Unit focused on generating and characterising mouse models for COVID disease.

The laboratory mouse is the most widely used animal model in biomedicine, but it is not a permissive species for SARS-CoV-2 infection. Structural differences between the human Angiotensin Converting Enzyme-2 (ACE2) protein, the cellular receptor for SARS-CoV-2, and its murine ortholog are the cause, at least in part, of the different sensitivity to viral infection in humans and mice. Supported by a dedicated grant from the Spanish Institute of Health *Carlos III* and a *SINERGIAS*-grant from the Madrid Local Government (CAM), the Unit has created "humanized" mouse models for the study of COVID19, in collaboration with the company Gen-H Genetic Engineering, Heidelberg (Germany).

Using the latest gene editing technologies, based on the CRISPR/Cas9 system, we created knockin mice in which the human ACE2 protein is expressed under the transcriptional control of the endogenous mouse *Ace2* promoter, interrupting simultaneously the *Ace2* coding sequence and resulting in the knockout of the mouse *Ace2* gene. As an alternative, we used a BAC transgene approach to drive expression of human ACE2 under the control of the *Ace2* promoter. In both cases, the expression of human ACE2 recapitulates the pattern and regulation of endogenous Ace2 expression.

During 2021, in collaboration with the Coronavirus Laboratory directed by Dr Luis Enjuanes at the *Centro Nacional de Biotecnología* (*CNB/CSIC*) in Madrid, we tested the susceptibility of our mouse models to SARS-CoV-2 infection. Intranasal inoculation of SARS-CoV-2 in the humanized knockin mice resulted in the accumulation of inflammatory infiltrate (CD45+ cells) surrounding lung alveoli and neighbouring blood vessels (CD31+) close to the cells where the human ACE2 protein is expressed in these mice (FIGURE 1), hence showing that our models are susceptible to SARS-CoV-2 infection. ■

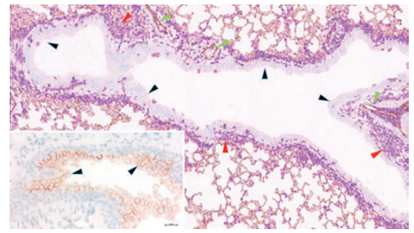

FIGURE 1 Lung inflammation in human ACE2 (hACE2) knockin mice, inoculated with SARS-CoV-2. CD45+ inflammatory cells (magenta; red arrowheads) and CD31+ endothelial cells (brown; green arrowheads) in the lung of a knockin mouse, 3 days after intranasal virus inoculation. Insert image: hACE2+ expressing cells (black arrowheads in both images) lining the bronchioles.

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