The CRISPR-Cas9 Minipig

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The pig is a preferred model for many diseases but studies have been hindered by time and budget limitations. We have cloned a transgenic minipig expressing Cas9 under a ubiquitous promoter, that substantially will reduce the cost and time to develop new pig models with unique gene alterations. This was carried out by inserting a transposon harboring the CRISPR-Cas9 gene into the genome of minipig fibroblasts. Fibroblasts containing few copies of the Cas9 transgene were selected and used for cloning by somatic cell nuclear transfer, which gave rise to seven founder pigs. The genetic design has been validated *in vitro* in fibroblasts isolated from the cloned Cas9 minipigs, where Sanger sequencing of DNA from fibroblasts transfected with guide RNAs against *TP53* and *PTEN* revealed knockout of the genes. Moreover, transfection with guide RNA against *KRAS* in combination with a homology directed repair templated revealed the desired G12D point-mutation leading to constative activation of KRAS.

IVIS scanning and IHC staining of tissues sections from one of the founder pigs verified transgene expression in major organs including heart, lung, liver, colon, and prostate. However, expression in hematopoietic cells could not be confirmed. The transgene expression varied from 25 to 100 percent positive cells among different organs and cell types.

A virus-based technology will be implemented for delivery of guide RNAs to induce gene alteration(s*) in vivo*. First, a pilot study in Danish Landrace pigs has validated that our adeno-associated virus particles indeed are capable of infecting the lung epithelium of pigs. The porcine Cas9 model is currently being validated *in vivo* by induction of lung cancer through mutation of *TP53*, *PTEN*, and *KRAS*. Here it is expected that a few cells (25-200) will be mutated in all three genes and that these will clonally expand to develop adenomas in the lung.

In conclusion, the development of the Cas9 minipig will open a new research area in minipigs, where gene alterations can be induced rapidly at a reduced cost. This will provide new models to study human diseases in a larger animal.

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