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GENETIC ENGINEERING

Pigs without PERVs

Pig-to-human organ transplantation holds promise for resolving the existing shortage of transplantable organs, but the potential transmission risk of porcine endogenous retroviruses (PERVs) to humans has remained a concern. Now, Niu *et al*. report the successful generation of PERV-inactivated pigs by somatic cell nuclear transfer (SCNT) from primary pig fetal fibroblast cells in which PERVs have been inactivated genome-wide using the CRISPR–Cas9 system.

In vitro studies have previously shown that PERVs can infect human cells where they integrate into the genome, although it remains unknown whether PERVs are transmitted to humans *in vivo*. Using droplet digital PCR on the reverse transcriptase (*pol*) gene in primary pig fibroblast cells, the authors identified 25 copies of functional PERVs and mapped these by hybridization capture followed by sequencing to their genomic locations. The team then designed two CRISPR guide RNAs (gRNAs) targeting the catalytic core of the PERV *pol* gene and transfected these into fibroblast cells together with Cas9 for 12 days. Culturing with a combination of apoptosis inhibitor and growth factor increased the average targeting efficiency and enabled the growth of 100% PERV-inactivated clones from the CRISPR–Cas9-treated cells. RNA sequencing confirmed the absence of *pol* transcripts, and no reverse transcriptase activity of PERVs could be detected in the cell culture supernatant, indicating successful PERV inactivation.

After selecting clones without detectable structural variations, PERV-inactivated embryos were generated by SCNT. Deep sequencing of the *pol* gene in embryos on day 6 and fetuses at pregnancy day 50 confirmed 100% PERV inactivation. Transgenic pigs were obtained at similar efficiency from PERV-inactivated cells and from wild-type cells. Sequencing of genomic DNA and RNA derived from these pigs showed stable PERV inactivation, ruling out PERV reinfection from surrogate sows to cloned pigs.

Given that Nui *et al*. also show that PERV-infected human cells transmit PERVs to other human cells with no prior exposure to pig cells, this study highlights the risk of cross-species viral transmission in the context of xenotransplantation and the value of PERV inactivation. Aside from the proof-of-principle that PERV inactivation is feasible *in vivo*, this foundational PERV-inactivated pig strain may be a useful resource for further genetic engineering to provide organs and tissues for human transplantation.

Linda Koch

> “successful generation of PERV-inactivated pigs by somatic cell nuclear transfer”

ORIGINAL ARTICLE Niu, D. *et al*. Inactivation of porcine endogenous retrovirus in pigs using CRISPR–Cas9. *Science* http://dx.doi.org/10.1126/science.aan4187 (2017)
FURTHER READING Yang, J. Y. C. & Sarwal, M. M. Transplant genetics and genomics. *Nat. Rev. Genet*. **18**, 309–326 (2017)