

Gene Editing Could Help Pave the Way for Pig-to-Human Transplantations

Tracy Hampton, PhD

Although much of the promise of CRISPR-Cas9 gene editing techniques has centered around correcting disease-causing gene mutations, the advance is also showing potential in the field of xenotransplantation, for example, for making porcine organs safe for transplantation into humans. In an important step in this direction, a team led by researchers at the biotechnology company eGenesis recently edited the pig genome to deactivate a family of retroviruses.

Organs from pigs, which can grow to a convenient human size, could help alleviate the growing organ shortage if scientists continue to make strides in addressing their immunological incompatibility; however, the pig genome includes remnants of ancient viral infections called porcine endogenous retroviruses (PERVs), which can be passed on to other cells when cultured together. In contrast to human endogenous retroviruses, which are mostly defective and not replication competent, PERVs that are integrated in the pig genome can be released as particles that infect human cells. As reported with other retroviruses, PERV integration could potentially lead to immunodeficiency and tumorigenesis. There may be an especially high risk of PERV transmission in transplant recipients who often require lifelong treatment with immunosuppressive drugs to prevent organ rejection.

Gene editing efforts by the eGenesis team have removed PERVs in



There is potential for the organs from pigs to help alleviate the growing organ shortage.

cell lines but not live pigs. In their previous [research](#) published in *Science*, the investigators determined the PERV copy number in a porcine kidney epithelial cell line to be 62. Next, they used the CRISPR (clustered, regularly interspaced, palindromic repeats) technology, which is adapted from a bacterial defense system and uses the DNA-cutting enzyme Cas9 bound to a guide RNA molecule that targets a specific genetic sequence. CRISPR-Cas9 allowed them to disrupt all 62 copies of the PERV *pol* gene. This “multiplexability” was a significant accomplishment compared with simpler gene editing feats, highlighting the precise yet widespread genetic changes possible through CRISPR-Cas9 editing. The modifications led to a >1000-fold reduction in PERV transmission to human cells from the engineered cells.

In the group’s latest study published in *Science*, cell culture experi-

ments confirmed that PERVs in pig cells can be transmitted to human cells and went a step further to demonstrate that the viruses can pass from human cells to other human cells. Although it unclear whether PERVs infect humans in vivo, these findings substantiate the risk of cross-species viral transmission with xenotransplantation.

As they did previously with kidney epithelial cells, the investigators mapped and characterized the PERVs present in the genome of porcine fetal fibroblast cells, this time identifying 25 viral copies. Next, they tailored the CRISPR-Cas9 technology to target the viral genomic sites they identified and to inactivate the PERVs within the fibroblasts.

Despite the presence of highly modified cells in the population, none of the targeted cells could be grown with >90% PERV editing efficiency. The scientists suspected that the simultaneous DNA cuts by Cas9

at multiple PERV sites triggered DNA damage–induced senescence or cell death. By adding a cocktail of factors that inhibit apoptosis and promote growth, the scientists were able to cultivate viable cells with 100% of PERVs inactivated.

Next, the team used the modified fibroblasts to produce PERV-inactivated embryos via somatic cell nuclear transfer and implanted the embryos into sows. The ratios of piglets born to number of embryos transferred were similar for PERV-inactivated cells (0.9%) and wild-type cells (0.8%). The resulting piglets exhibited no signs of PERVs or abnormal structural changes to chromosomes. The investigators have produced 37 PERV-inactivated piglets from 17 sows (200–300 em-

bryos transferred per sow). Fifteen piglets remain alive, and the oldest healthy animals are 4 months old. The authors noted that these piglets are the first animals born free of endogenous virus. They plan to conduct long-term studies to monitor the impact of PERV inactivation and gene editing as the animals age.

“This research represents an important advance in addressing safety concerns about cross-species viral transmission. Our team will further engineer the PERV-free pig strain to deliver safe and effective xenotransplantation,” said senior author Luhan Yang, PhD, who is cofounder of and chief scientific officer at eGenesis.

In addition to raising the prospect of xenotransplantation, the research likely has applications for modifying

other genetic repetitive elements of biological interest. Studies are even pointing to the possibility of harnessing CRISPR-Cas9 to target HIV, potentially shutting down replication and eliminating the virus from infected cells. Much work remains, however, because time and again HIV has demonstrated an ability to evolve resistance to all manner of attacks.

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