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Pig-to-Human xenotransplantation: Moving toward organ customization

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Dear Editor,

Xenotransplantation is a potentially effective way to address the shortage of organ donors. The improvements in gene editing technology have accelerated a number of a breakthrough in genetically modifying pigs for xenotransplantation. In 2022, xenograft kidney transplantation entered the subclinical trial stage. Two teams independently transplanted genetically modified porcine kidneys into brain-dead patients. The porcine kidneys remained viable and functional in the recipients for 54 hours and 74 hours, respectively, with no significant signs of acute rejection.[1,2] Notably, the University of Maryland Medical Center completed the first pig-to-human heart transplant on January 7, 2022, successfully transplanting a pig heart that had 10 gene edits into a patient with end-stage heart failure, who survived 60 days following surgery.[3] This series of milestone scientific studies represents a revolutionary breakthrough in the clinical research of xenotransplantation.

Depending on the varied target organs, xenotransplantation faces a diverse set of physiological challenges. Gene editing technology enables xenotransplantation to move toward customization (Fig. 1). Here we summarize precision strategies in light of the latest gene-edited pig-to-primate organ xenotransplantation studies.

In the first pig-to-human cardiac xenotransplantation, the glycosylation antigen genes alpha-1,3-galactosyltransferase (*GGTA1*) which causes hyperacute rejection (HAR), cytidine monophosphate-N-acid hydroxylase (*CMAH*)/*β1*, 4N-acetylgalactosaminyltransferase (*βGalNT2*), which cause acute humoral xenograft rejection (AXHR) were knocked out; and human CD47 (*hCD47*) gene, which prevents acute cellular xenograft rejection (ACXR), was inserted. Gene editing that express human CD46 and attenuation acceleration factor (CD55) helps to reduce antibody-dependent complement transplantation damage. To enhance the inefficiency of porcine-derived blood factors to activate protein C, the human thrombomodulin (TBM) and endothelial cell protein C receptor (EPCR) were also expressed. The anti-inflammatory protein heme oxygenase-1 (HO-1) was also expressed. To stop xenografts from overgrowing, the growth hormone receptor (*GHR*) gene was also knocked out. Unexpectedly, the patient survived two months following surgery, which is a triumph for xenotransplantation. However, the porcine cytomegalovirus (pCMV), which was neglected in preoperative assays, was unexpectedly present in the transplanted pig heart. Therefore, concerns lie not only in which pig breeds are ideal for xenotransplantation, but also in how to guarantee they are free of virus. In addition, the maladaptive hypertrophy of the heart that causes diastolic heart failure may also be a hazard; pretransplant nonischemic preservation strategies are also needed.[4,5]

In 2022, researchers transplanted two kidneys from a pig with 10 gene edits into brain-dead patients.[1] The transplanted porcine kidneys produced urine and showed no significant HAR in the 74-hour experiment. However, it failed to clear creatinine and suffered by thrombotic microangiopathy. Another team transplanted kidneys with the *GGTA1* gene knocked out and with subcapsular autologous thymic tissue into two brain-dead human recipients. The xenograft in both recipients started producing urine shortly after reperfusion, and the porcine kidneys remained viable and functioning in human recipients for 54 hours, without showing HAR symptoms.[2] Besides HAR, the hypovolemia syndrome and erythropoietin function-associated anemia after transplantation are also important organ-specific issues in kidney xenotransplantation, which should be solved via genetically engineering pig kidneys to express humanized erthropoietin.

Pig liver xenotransplantation is more complex to perform than heart and kidney xenotransplantation. Thrombotic microangiopathy and systemic consumptive coagulopathy seem to be severe after pig liver xenotransplantation. Since 2010, the use of *GGTA1* knockout (GTKO) and GTKO/hCD49 pigs for liver xenografts has extended graft survival time to 9 days.[5,6] According to these studies, the key factor limiting liver graft survival is coagulopathy. The researchers then used a combination of costimulatory blockers, anti-CD40 mAb, to extend the time to maintain liver function in baboons to 29 days, which was the longest survival time of liver xenotransplantation from pigs-to-nonhuman primates (NHPs).[7] Thus, in liver xenotransplantation, how to prevent coagulation dysregulation and allow spontaneous platelet count recovery is a key issue to be addressed.

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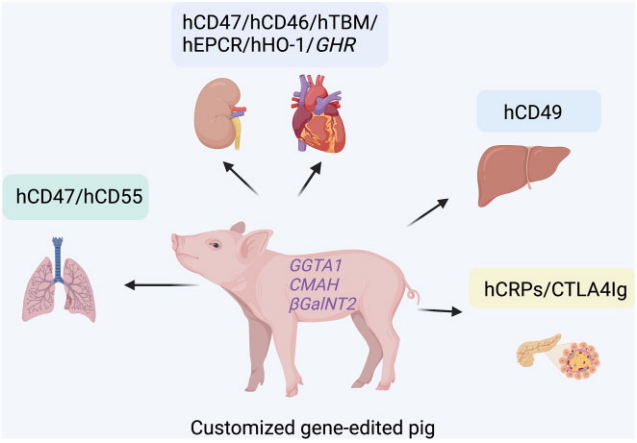

Figure 1. Customized gene editing strategies for different organs in xenotransplantation.

Rapid coagulation disorder is a major problem for lung transplantation. Using GTKO/hCD47/hCD55 transgenic pigs can only extend the survival time of NHP recipients up to 14 days.[8] The limited survival time suggests the necessity of additional coagulation dysregulation strategies in lung xenotransplantation.

For islet transplantation, persistent innate inflammatory response limits the survival of porcine islets in NHPs. Human complement regulatory proteins, CTLA4Ig, and anticoagulant protein transgenes may be important in controlling the immediate innate immune response.[9] The adult porcine islets do not express α-Gal, so *GGTA1* knockout is less necessary in islet transplantation.[10]

Despite all the benefits of gene editing for xenotransplantation, excessive gene editing, however, not only lowers the survival probability of the donor pig, but also raises the exposure to additional xenoantigens of the recipient. To improve the physiological functional compatibility of grafts, screening and assessing suitable donor gene-editing combinations are needed in clinical organ xenotransplantation. In addition to the urgent problems of immune incompatibility, functional compatibility, and safety hazards of cross-species transmission, selection of appropriate recipients for various transplant organ types is also essential in xenotransplantation clinical trials. In addition, clinical guidelines for allogeneic organ transplantation need to be further refined and clarified for enhanced public acceptance.

It is anticipated that more studies on xenotransplantation will soon be conducted to jointly promote precision organ xenotransplantation from the experimental stage to clinical application. Xenotransplantation calls for exploration of safe and effective gene editing technology, development of new immunosuppressants, discovery of new heterogenetic antigens, accumulation of experience in preclinical studies, and improvement of relevant laws and regulations.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Porrett PM, Orandi BJ, Kumar V, *et al.* First clinical-grade porcine kidney xenotransplant using a human decedent model. *Am J Transplant* 2022; **22**: 1037–53. doi:10.1111/ajt.16930
2. Montgomery RA, Stern JM, Lonze BE, *et al.* Results of two cases of pig-to-Human kidney xenotransplantation. *N Engl J Med* 2022; **386**: 1889–98. doi:10.1056/NEJMoa2120238
3. Griffith BP, Goerlich CE, Singh AK, *et al.* Genetically modified porcine-to-Human cardiac xenotransplantation. *N Engl J Med* 2022; **387**: 35–44. doi:10.1056/NEJMoa2201422
4. Längin M, Mayr T, Reichart B, *et al.* Consistent success in life-supporting porcine cardiac xenotransplantation. *Nature* 2018; **564**: 430–3. doi:10.1038/s41586-018-0765-z
5. Ekser B, Long C, Echeverri GJ, *et al.* Impact of thrombocytopenia on survival of baboons with genetically modified pig liver transplants: Clinical relevance. *Am J Transplant* 2010; **10**: 273–85. doi:10.1111/j.1600-6143.2009.02945.x
6. Kim K, Schuetz C, Elias N, *et al.* Up to 9-day survival and control of thrombocytopenia following alpha1,3-galactosyl transferase knockout swine liver xenotransplantation in baboons. *Xenotransplantation* 2012; **19**: 256–64. doi:10.1111/j.1399-3089.2012.00717.x
7. Shah JA, Patel MS, Elias N, *et al.* Prolonged survival following pig-to-primate liver xenotransplantation utilizing exogenous coagulation factors and costimulation blockade. *Am J Transplant* 2017; **17**: 2178–85. doi:10.1111/ajt.14341
8. Watanabe H, Ariyoshi Y, Pomposelli T, *et al.* Intra-bone bone marrow transplantation from hCD47 transgenic pigs to baboons prolongs chimerism to >60 days and promotes increased porcine lung transplant survival. *Xenotransplantation* 2020; **27**: e12552. doi:10.1111/xen.12552
9. Graham ML, Ramachandran S, Singh A, *et al.* Clinically available immunosuppression averts rejection but not systemic inflammation after porcine islet xenotransplant in cynomolgus macaques. *Am J Transplant* 2022; **22**: 745–60. doi:10.1111/ajt.16876
10. Dor FJ, Cheng J, Alt A, *et al.* Gal alpha 1,3Gal expression on porcine pancreatic islets, testis, spleen, and thymus. *Xenotransplantation* 2004; **11**: 101–6. doi:10.1111/j.1399-3089.2004.00078.x

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