

Translational Research Urology

Home Page: www.transresurology.com

Editorial

Beyond Science Fiction: Xenotransplantation Becoming Clinical Reality

Seyedeh Negin Hashemi Dougaheh

Department of Stem Cells Technology and Tissue Regeneration, School of Biology, College of Science, University of Tehran, Tehran, Iran

HIGHLIGHTS

- Genome editing approaches can modify pig's organs to be inert and efficient.
- Gene knockouts of porcine surface markers can reduce the risk of HAR and acute immune response.
- Human transgenes can be inserted to express self-antigens and control complement response.

ARTICLE INFO

Receive Date: 23 August 2023

Accept Date: 26 October 2023

Available online: 30 October 2023

DOI: 10.22034/TRU.2023.421973.1163

*Corresponding Author:

Seyedeh Negin Hashemi Dougaheh

Email: snhd.nh@gmail.com

Address: Department of Stem Cells Technology and Tissue Regeneration, School of Biology, College of Science, University of Tehran, Tehran, Iran

ABSTRACT

The growing demand for organ transplants has led to a shortage of available organs. This shortage has contributed to crises such as illegal organ trafficking and transplant tourism. However, recent progress in xenotransplantation may help alleviate this shortage. With current successes in transplanting pig organs into non-human primate models and deceased patients, companies are spending a great deal of money on establishing pathogen-free pig facilities and gaining FDA approval to move toward clinical trials. Given this progress, it is worthwhile to examine the current state of xenotransplantation and ongoing approaches to modifying porcine organs to make them safe for clinical use in humans.

Keywords: Thymokidney; CRISPER-Cas9; Transplantation; Immune Response

Editorial: From the first kidney transplants from chimpanzees (Dr. Reemtsma, 1963) and baboons (Dr. Starzl, 1964) onwards, the field of xenotransplantation has wavered greatly between showing enormous promise and facing seemingly impossible hurdles. However, the evolution of gene editing techniques like Cre-mediated cassette exchange, TALEN, zinc finger nuclease, and CRISPR-Cas9 has now made it possible to move beyond apes and genetically modify other animals' organs to better fit human needs. Pigs have currently replaced apes as the focus of xenotransplantation research, as pigs are less endangered, have shorter gestation periods and higher litter numbers, and possess organs with similar physiological

properties and comparable size to human organs.

Among the different approaches towards xenotransplantation, such as using acellular porcine organs as a scaffold for human kidney cells or blastocyst complementation to grow new human kidneys in pigs, using genetically edited pig kidneys has been the most studied approach and shows the most promising results. This is because we now have a solid understanding of the cells and molecules responsible for transplant rejection, as well as ways of editing them.

The first pitfall in xenotransplantation that arises minutes to hours after transplantation is hyperacute rejection (HAR). This response is mediated by pre-

existing antibodies in human blood that bind to glycoproteins on the surface of porcine cells. To address this, the GGTA1 gene is typically knocked out in the germline of pig donors as a first step. GGTA1 encodes an enzyme called α -1,3-galactosyltransferase that places α -gal epitopes on cell surface glycoproteins. Humans and apes do not express this gene and have abundant neutralizing antibodies against it since it is highly prevalent on bacterial surfaces. In addition to GGTA1 knockout (1), recent publications indicate that knocking out the CMAH and B4GALNT2/B4GALNT2L genes (3KO model) is also required to prevent any form of humoral response in humans.

Antibody recognition of cell surface markers also leads to instant blood-mediated inflammatory reaction (IBMIR), a nonspecific response involving complement activation, innate immunity, and coagulation dysfunction. Coagulation occurs due to porcine cells' inability to produce sufficient active protein C, leading to hypercoagulation. Inserting tissue factor pathway inhibitor (TFPI)-1, CD141 (thrombomodulin), and CD201 (human endothelial protein C receptor) can promote more efficient protein C activation. Additionally, inserting CD39 and CD73 and inducing von Willebrand factor (vWF) deficiency prevents platelet adhesion and coagulation. Without coagulation, the risk of innate immune cell activation is reduced since coagulates act as damage-associated molecular patterns (DAMPs) that trigger inflammation (2).

While the absence of antibody attachment after removing cell surface markers reduces complement deposition, inserting human transgenes such as CD46 (membrane cofactor protein), CD55 (decay-accelerating factor), CD59 (membrane attack complex inhibitor), or combinations thereof, can further attenuate complement cascade activation (3).

α -gal elimination also prevents antibody-dependent cell-mediated cytotoxicity (ADCC) by NK cells. However, ensuring the absence of innate immune responses can be achieved by inserting human self-

antigens into the pig genome or eliminating the pig's antigen-presenting molecules. Knockout candidates include β 2M and CIITA, activating MHC class I and II, respectively. Inserting human CD47, recognized as a self-antigen by macrophages' SIRP α receptor, into the pig genome can also help evade innate immune detection (4).

Transplanting CD47 and β 2M or CIITA removal, alongside immunosuppressive regimens prescribed for transplant patients (such as anti-CD154, mycophenolate mofetil, and steroids) helps reduce T cell activation. As B cell function largely relies on T cell help, this also attenuates further humoral rejection (5). Another approach to decrease acute cellular rejection involves using thymokidneys - xenotransplanted kidneys with preceding subcapsular autologous implantation of the vascularized minced thymus. This partial thymus is thought to induce regulatory T cells to prevent subsequent T cell responses against the exogenous kidney (6). However, more precise gene editing to reduce Fas ligand and increase PD-L1 or CTLA4 expression in pigs may be a more plausible strategy (1).

While the earlier approaches prevent acute rejection of xenotransplants, concerns remain about porcine endogenous retroviruses (PERVs). PERVs are common γ -retroviruses integrated into the porcine genome. They can produce viable virion particles, and as they have a tropism towards human cells, they can also induce leukemia and immunodeficiency. Although efficient vaccines exist for related γ -retroviruses, PERV genes can also be knocked down using indel mutations (7).

Conclusion

Recent advances in genome editing methods such as CRISPER-Cas9 have brought xenotransplantation closer to the clinic. While challenges around rejection and infections persist, targeted multi-gene knockouts or human transgene insertions can provide a path forward to address the organ shortage crises.

Table 1. Advancements in kidney xenograft

Xenotransplant	Edited genes	Recipients	Longest reported survival	Complementation treatments	Ref
Thymokidney (provided by Revivicore)	α -gal knock out	Brain dead human	> 61 days	Methylprednisolone, mycophenolate mofetil	1
Genetically edited xenokidney	69 edited genes: 3KO (GGTA1, CMAH, and B4GALNT2/B4GALNT2L), 7 human transgenes: CD46 and CD55 – THBD and PROCR – CD47 – TNFAIP3 and HMOX1, 59 KO for PERV	Cynomolgus monkey	> 758 days	Immunosuppression of B and T cells, anti CD154 mAb, mycophenolate mofetil, and short-term tacrolimus and steroids	3
Genetically edited xenokidney	α -gal knock out – CD55 transgene – α CD8+ depletion, α CD4+ depletion, or both	Rhesus macaques	> 499 days	Mycophenolate mofetil, solumedrol taper, and α CD154 (5c8)	5

Authors' contributions

Not Applicable.

Acknowledgments

Not Applicable.

Conflict of interest

The author declares that there is no conflict of interest.

Funding

There is no funding.

Ethics statement

Not Applicable.

Data availability

None.

Abbreviations

ADCC	Anti-body Dependent Cell Mediated Cytotoxicity
Cas9	CRISPER-Associated protein9
CIITA	MHC Class II Trans Activator
CRISPER	Clustered Regulatory Interspaced Short Palindromic Repeats
DAMP	Damage Associated Molecular Pattern
HAR	Hyper Acute Rejection
IBMIR	Instant blood-mediated Inflammatory Reaction
PERV	Porcine endogenous retrovirus
TALEN	Transcription activator-like effector nuclease
Vwf	Von Willebrand factor
α -gal	Galactose- α -1,3-galactose

References

1. Montgomery RA, Stern JM, Lonze BE, Tatapudi VS, Mangiola M, Wu M, et al. Results of two cases of pig-to-human kidney xenotransplantation. *New England Journal of Medicine*. 2022;386(20):1889-98.
2. Meier RP, Muller YD, Balaphas A, Morel P, Pascual M, Seebach JD, et al. Xenotransplantation: back to the future? *Transplant International*. 2018;31(5):465-77.
3. Anand RP, Loyer JV, Heja D, Hirose T, Lassiter G, Firl DJ, et al. Design and testing of a humanized porcine donor for xenotransplantation. *Nature*. 2023;622(7982):393-401.
4. Carvalho-Oliveira M, Valdivia E, Blasczyk R, Figueiredo C. Immunogenetics of xenotransplantation. *International Journal of Immunogenetics*. 2021;48(2):120-34.
5. Kim SC, Mathews DV, Breeden CP, Higginbotham LB, Ladowski J, Martens G, et al. Long-term survival of pig-to-rhesus macaque renal xenografts is dependent on CD4 T cell depletion. *American Journal of Transplantation*. 2019;19(8):2174-85.
6. Yamada K, Ariyoshi Y, Pomposelli T, Sekijima M. Co-transplantation of vascularized thymic graft with kidney in pig-to-nonhuman primates for the induction of tolerance across xenogeneic barriers. *Xenotransplantation: Methods and Protocols*. 2020:151-71.
7. Mohammadi B, Esmailizadeh Z, Rajabibazl M, Ghaderian SMH, Omrani MD, Fazeli Z. Preconditioning of human adipose tissue-derived mesenchymal stem cells with HEK293-coditioned

Author (s) biosketches

Hashemi Dougaheh SN, MSc, Department of Stem Cells Technology and Tissue Regeneration, School of Biology, College of Science, University of Tehran, Tehran, Iran.
Email: snhd.nh@gmail.com

How to cite this article

Hashemi Dougaheh SN. Beyond Science Fiction: Xenotransplantation Becoming Clinical Reality. *Translational Research in Urology*. 2023 Nov 5(4): 147-149.

DOI: [10.22034/TRU.2023.421973.1163](https://doi.org/10.22034/TRU.2023.421973.1163)

URL: https://www.transresurology.com/article_181871.html

