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Engineering Immune-Compatible Organs: Genetic Modifications in Pigs for Reduced Rejection in Human Recipients

Xiaofang Lin
Tropical Animal Medicine Center, Hainan Institute of Tropical Agricultural Resources, Sanya, 572024, Hainan, China
Corresponding author: xiao'fanglin@hitar.org

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Abstract The shortage of human organs for transplantation has driven significant advancements in xenotransplantation, particularly using genetically modified pigs. This study examines the genetic modifications in pigs aimed at reducing immune rejection in human recipients. Recent studies have demonstrated the potential of porcine organs with multiple genetic modifications to overcome hyperacute rejection and improve graft survival. Key genetic alterations include the knockout of xenoantigens such as alpha-1,3-galactosyltransferase and the insertion of human complement and coagulation regulatory genes. These modifications have shown promising results in preclinical and early clinical trials, with some xenografts maintaining function without signs of rejection for extended periods. The study highlights the importance of continued research to optimize genetic modifications and address remaining immunological and physiological barriers to clinical xenotransplantation.

Keywords Porcine organs; Xenotransplantation; Genetic modification; Immune rejection; CRISPR-Cas9

Organ transplantation has long been recognized as a life-saving treatment for patients with end-stage organ failure. However, the demand for human organs far exceeds the supply, leading to a significant shortage that results in the deaths of thousands of patients annually while they await transplants (Sykes and Sachs, 2019; Lu et al., 2020). This critical imbalance has driven the exploration of alternative sources of organs, including xenotransplantation, which involves the transplantation of organs from one species to another (Yue et al., 2020; Lei et al., 2022).

Xenotransplantation, particularly using pigs as organ donors, has emerged as a promising solution to the organ shortage crisis. Pigs are considered suitable donors due to their physiological similarities to humans and their ability to be genetically modified to reduce immunological incompatibilities (Cooper et al., 2019; Xi et al., 2023). Recent advancements in genetic engineering, such as CRISPR-Cas9, have enabled the creation of pigs with multiple genetic modifications aimed at overcoming the major barriers to xenotransplantation, including hyperacute rejection and other immune responses (Sykes and Sachs, 2019; Yue et al., 2020). These modifications include the deletion of pig-specific antigens and the expression of human complement and coagulation regulatory proteins (Cooper et al., 2019; Wu et al., 2023).

The primary objective of this study is to examine the various genetic modifications in pigs that have been developed to reduce the risk of organ rejection in human recipients. By systematically analyzing the current state of research, this study aims to highlight the most effective genetic strategies that have been employed to enhance the compatibility of pig organs with the human immune system. Understanding these genetic modifications is crucial for advancing the field of xenotransplantation and moving closer to clinical applications that could alleviate the organ shortage crisis. This review will also discuss the potential challenges and future directions in the genetic engineering of pigs for xenotransplantation, providing a comprehensive overview of the progress and prospects in this innovative field.

By addressing these objectives, this study seeks to contribute to the ongoing efforts to develop viable and immune-compatible pig organs for human transplantation, ultimately improving patient outcomes and saving lives.

1 The Need for Immune-Compatible Organs

1.1 Challenges of organ rejection in transplantation

Organ transplantation has been a critical medical advancement for treating end-stage organ failure. However, one of the most significant challenges in transplantation is the risk of organ rejection, which can be acute, chronic, or hyperacute. Rejection occurs when the recipient's immune system recognizes the transplanted organ as foreign and mounts an immune response against it. This immune response can be mediated by T-cells, antibodies, or innate immune cells such as macrophages and natural killer (NK) cells (Yılmaz et al., 2020; Nguyen et al., 2021; Lu et al., 2022). The use of immune checkpoint inhibitors (ICI) has shown clinical benefits in cancer patients but has also been associated with increased risks of transplant rejection, particularly in kidney and liver transplant recipients (Nguyen et al., 2021).

1.2 Importance of immune compatibility in xenotransplantation

Xenotransplantation, the transplantation of organs from one species to another, has emerged as a promising solution to the shortage of human organs available for transplantation. Pigs are considered ideal donors due to their physiological similarities to humans and the feasibility of genetic modifications. However, the genetic and molecular incompatibilities between pigs and humans pose significant barriers, leading to xenogeneic rejection (Sykes and Sachs, 2019; Yılmaz et al., 2020; Lu et al., 2022). Innate immune responses, including those mediated by macrophages, NK cells, and neutrophils, play a crucial role in xenogeneic rejection (Maeda et al., 2020; Lu et al., 2022). Addressing these immune compatibility issues is essential for the success of xenotransplantation.

1.3 Current strategies to address organ rejection

Several strategies have been developed to mitigate the risk of organ rejection in both allotransplantation and xenotransplantation. These include the use of immunosuppressive therapies, genetic modifications, and pretransplant desensitization techniques. Advances in gene-editing technologies, such as CRISPR-Cas9, have enabled the creation of genetically modified pigs with reduced expression of antigens that trigger immune responses, such as galactose-α1,3-galactose and N-glycolylneuraminic acid (Sykes and Sachs, 2019; Yılmaz et al., 2020). Additionally, overexpression of inhibitory ligands on porcine cells has been shown to suppress macrophage-mediated rejection (Maeda et al., 2020). Understanding the mechanisms of innate immune responses and developing targeted therapies to modulate these responses are critical for improving the outcomes of xenotransplantation.

In conclusion, while significant progress has been made in addressing the challenges of organ rejection, ongoing research and development of innovative strategies are essential to achieve immune-compatible organs for transplantation. The integration of genetic modifications and advanced immunosuppressive therapies holds promise for the future of xenotransplantation and the potential to save countless lives.

2 Genetic Basis of Immune Rejection

2.1 Overview of the human immune response to foreign organs

The human immune system is highly adept at recognizing and responding to foreign tissues, a process that is critical in the context of organ transplantation. The primary immune response to transplanted organs involves both the innate and adaptive immune systems. The innate immune response is the first line of defense and includes mechanisms such as inflammation and the activation of macrophages and natural killer (NK) cells (Figure 1) (Ravichandran et al., 2022; Zhang et al., 2022). The adaptive immune response, which is more specific and involves memory, is mediated by T and B lymphocytes. T cells recognize foreign antigens presented by major histocompatibility complex (MHC) molecules on the surface of donor cells, leading to T-cell activation and proliferation (Ronca et al., 2020; Morazán-Fernández et al., 2022). B cells produce antibodies against donor antigens, contributing to antibody-mediated rejection (ABMR) (Yazdani et al., 2019; Morazán-Fernández et al., 2022).

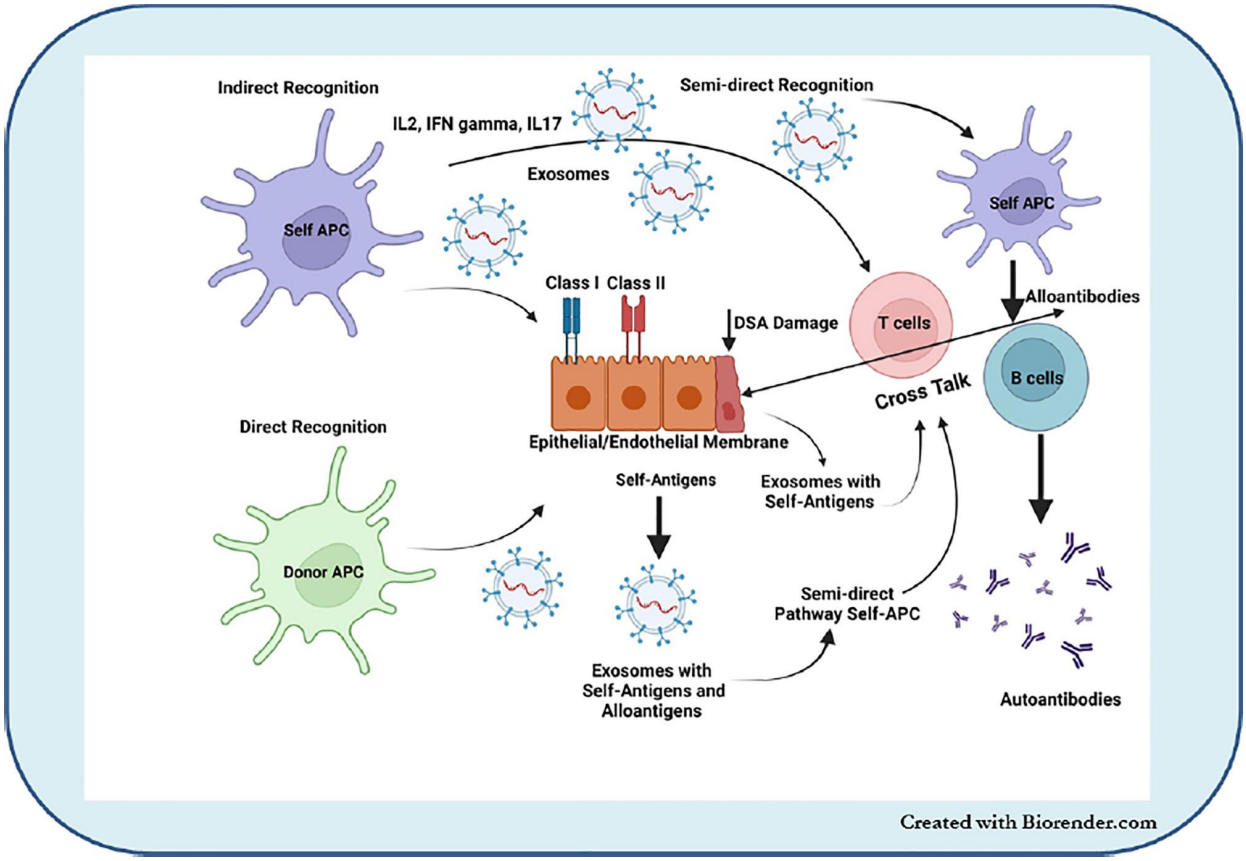

Figure 1 EVs interact with cells via numerous ligand–receptor interactions (Adopted from Ravichandran et al., 2022)
Image caption: sEVs can activate not only direct and indirect pathways of antigen presentation but also via the semidirect pathway in which T cell activation occurs via donor-derived sEVs (Adopted from Ravichandran et al., 2022)

Ravichandran et al. (2022) shows the role of small extracellular vesicles (sEVs) in transplant rejection. sEVs, enriched with markers like CD9, CD63, and CD81, carry biomolecules specific to lung, heart, and kidney tissues. Lung sEVs with collagen type V and K alpha 1 tubulin indicate lung transplant rejection. Heart sEVs with myosin and vimentin and kidney sEVs with fibronectin and collagen IV are linked to heart and kidney transplant rejection, respectively. Monitoring these sEVs can provide early diagnostic markers for transplant rejection and aid in managing transplant patients.

2.2 Key genetic factors involved in immune rejection

Genetic differences between donor and recipient, particularly in the MHC or human leukocyte antigen (HLA) genes, are the primary cause of immune rejection. Variations in these genes lead to the recognition of the transplanted organ as foreign by the recipient's immune system (Morazán-Fernández et al., 2022). Specific genes and their associated pathways have been identified as critical in the rejection process. For instance, the expression of interferon-gamma (IFNG)-inducible genes such as *CXCL11* and *IDO1*, and genes associated with effector T cells and NK cells like *KLRD1* and *CCL4*, are strongly linked to rejection (Halloran et al., 2018). Additionally, genes involved in the inflammasome pathway, such as *AIM2*, have been implicated in acute rejection, highlighting their potential as therapeutic targets (Tejada et al., 2022). Other important genetic factors include polymorphisms in cytokine genes like *IL6*, which have been associated with varying risks of acute rejection (Hu et al., 2024).

2.3 Mechanisms of hyperacute, acute, and chronic rejection

Rejection of transplanted organs can occur at different stages, each with distinct mechanisms:

Hyperacute Rejection: This occurs within minutes to hours after transplantation and is primarily mediated by pre-existing antibodies in the recipient that recognize antigens on the donor organ. These antibodies activate the complement system, leading to rapid and severe damage to the graft (Morazán-Fernández et al., 2022).

Acute Rejection: This can occur days to weeks post-transplant and involves both T-cell-mediated rejection (TCMR) and antibody-mediated rejection (ABMR). TCMR is characterized by the direct attack of donor cells by recipient T cells, while ABMR involves the production of donor-specific antibodies that target the graft, leading to inflammation and tissue damage (Ronca et al., 2020; Teng et al., 2022). Genes such as *SLAMF8* and *TLR4* have been identified as playing roles in the inflammatory response during acute rejection.

Chronic Rejection: This occurs over months to years and is a major cause of long-term graft failure. Chronic rejection involves a combination of immune and non-immune factors, including continuous low-level immune responses and fibrosis. M2 macrophages, which are involved in tissue repair and fibrosis, play a significant role in chronic rejection by contributing to graft vasculopathy and fibrosis (Zhang et al., 2021). Additionally, extracellular vesicles released from the graft can mediate immune responses and contribute to chronic rejection by presenting donor antigens to the recipient's immune system (Ravichandran et al., 2022).

Understanding these mechanisms and the genetic factors involved is crucial for developing strategies to engineer immune-compatible organs and improve the outcomes of organ transplantation.

3 Genetic Modifications in Pigs to Reduce Rejection

3.1 Overview of genetic engineering techniques

Genetic engineering techniques such as CRISPR/Cas9 and TALENs have revolutionized the field of xenotransplantation by enabling precise modifications in the pig genome to reduce immunogenicity and improve compatibility with human recipients. CRISPR/Cas9, in particular, has been widely used due to its high efficiency and specificity. This technique involves the use of guide RNAs (gRNAs) to direct the Cas9 nuclease to specific genomic loci, where it introduces double-strand breaks that are repaired by non-homologous end joining or homology-directed repair, leading to targeted gene modifications (Zhang et al., 2018; Fu et al., 2020; Tanihara et al., 2021; Yoon et al., 2022). TALENs, another genome editing tool, use engineered nucleases to create double-strand breaks at specific sites, although they are less commonly used compared to CRISPR/Cas9 due to their complexity and lower efficiency (Yoon et al., 2022).

3.2 Specific genes targeted for modification

3.2.1 *GGTA1* (alpha-gal knockout) to prevent hyperacute rejection

The *GGTA1* gene encodes the enzyme α1,3-galactosyltransferase, which is responsible for the synthesis of the α-Gal epitope, a major xenoantigen that triggers hyperacute rejection in human recipients. Knockout of the *GGTA1* gene in pigs has been shown to significantly reduce the binding of human IgG and IgM antibodies, thereby preventing hyperacute rejection (Fu et al., 2020; Tanihara et al., 2021). Studies have demonstrated that *GGTA1* knockout pigs exhibit reduced expression of α-Gal in various tissues, including the heart, lungs, liver, and kidneys, making them more suitable for xenotransplantation (Wang et al., 2018; Zhang et al., 2018; Yoon et al., 2022).

3.2.2 *CMAH* and *β4GalNT2* to reduce xenoantigen expression

In addition to *GGTA1*, the *CMAH* and *β4GalNT2* genes are also targeted to reduce xenoantigen expression (Figure 2). The *CMAH* gene encodes CMP-Neu5Ac hydroxylase, which is involved in the synthesis of N-glycolylneuraminic acid (Neu5Gc), another xenoantigen that elicits immune responses in humans. Knockout of the *CMAH* gene in pigs has been shown to reduce Neu5Gc expression and decrease human antibody binding (Wang et al., 2018; Yoon et al., 2022). Similarly, the *β4GalNT2* gene encodes β-1,4-N-acetyl-galactosaminyl transferase 2, which is responsible for the synthesis of the Sd(a) antigen. Knockout of *β4GalNT2* in pigs further reduces xenoantigen expression and enhances immune compatibility (Zhang et al., 2018).

Tanihara et al. (2021) demonstrates the successful generation of gene-edited piglets using CRISPR/Cas9 technology targeting *GGTA1*, *CMAH*, and *β4GalNT2*. Two piglets (#4 and #5) were born from zygotes electroporated with Cas9 and guide RNAs. Deep sequencing revealed piglet #4 had biallelic mutations in *GGTA1* and *β4GalNT2*, while piglet #5 had mutations in all three target genes, including an inframe mutation in *β4GalNT2*. The high mutation frequencies and rates indicate effective gene editing. This study showcases the potential of CRISPR/Cas9 for creating genetically modified pigs for xenotransplantation research.

Piglet	Gene	Mutation		Genome sequence*	Indels	Frequency (%)**	Mutation rate (%)***
#4	*GGTA1*	Biallelic	target	gaAGACGCTATAGGCAACGAAAAGGaacaaa			
				gaagacgctataggca**c**--aaaaggaacaaa	−2bp, m1bp	15705/32359 (48.5)	31291/32359 (96.7)
				gaagacgctataggcaacg**g**aaaaggaacaaa	+1bp	15586/32359 (48.2)	
	CMAH	WT	target	ccGAAGCTGCCAATCTCAAGGAAGGaatcaa			
				ccgaagctgccaatctcaaggaaggaatcaa	none	34172/34758 (98.3)	–
	B4GALNT2	Biallelic	target	tcTTGAGGATCGACAGACATCTAGGgctgtt			
				tcttgaggatcgacaga**t**-**c**ctagggctgtt	−1bp, m2bp	32997/33964 (97.2)	32997/33964 (97.2)
#5	*GGTA1*	Biallelic	target	gaAGACGCTATAGGCAACGAAAAGGaacaaa			
				gaagacgctataggcaac----------aaa	−10bp	18016/34704 (51.9)	33807/34704 (97.4)
				gaagacgctataggcaacg**a**aaaaggaacaaa	+1bp	15791/34704 (45.5)	
	CMAH	Biallelic	target	ccGAAGCTGCCAATCTCAAGGAAGGaatcaa			
				ccgaagctgccaatctcaa**a**ggaaggaatcaa	+1bp	31026/31829 (97.5)	31026/31829 (97.5)
	B4GALNT2	Biallelic (with inframe)	target	tcTTGAGGATCGACAGACATCTAGGgctgtt			
				tcttgaggatcgacagacatctatcttgtgtcc**c**tagggctgtt	+13bp, m1bp	16137/32911 (49.0)	31632/32911 (96.1)
				tcttgaggatcgacagac**t**gggctgtctagggctgtt	+6bp, m1bp	15495/32911 (47.1)	

Figure 2 Deep sequencing analysis of the *GGTA1*, *CMAH*, and *B4GALNT2* target regions in delivered piglets (Adopted from Tanihara et al., 2021)

Image caption: * Blue and red indicate the target sequences and PAM sequences of each gRNA, respectively. Green and yellow indicate inserted and modified sequences, respectively. ** The frequency was defined as the ratio of the number of amplicons to the total read number. *** The mutation rate was defined as the ratio of the total number of mutant amplicons to the total read number. WT, wild-type. Underlining indicates the presence of an inframe mutation (Adopted from Tanihara et al., 2021)

3.2.3 Other modifications to enhance immune compatibility (e.g., CD47, HLA-E)

Beyond the knockout of xenoantigen genes, other genetic modifications have been explored to enhance immune compatibility. For instance, the overexpression of human CD47 in pigs has been investigated to inhibit phagocytosis by human macrophages, thereby reducing immune rejection (Fu et al., 2020). Additionally, modifications to the swine leukocyte antigen (SLA) genes, such as the knockout of β2-microglobulin (B2M) and the major histocompatibility complex class II transactivator (CIITA), have been shown to reduce the expression of SLA class I and class II molecules, respectively. This reduction in SLA expression decreases the activation of human T cells and prolongs the survival of pig xenografts in human recipients (Hein et al., 2019; Xu et al., 2022).

In summary, the use of advanced genetic engineering techniques like CRISPR/Cas9 has enabled the precise modification of specific genes in pigs to reduce xenoantigen expression and enhance immune compatibility, thereby improving the prospects of successful xenotransplantation.

4 Case Studies and Experimental Results

4.1 Notable experiments and their outcomes in reducing rejection

Several notable experiments have demonstrated significant progress in reducing xenograft rejection through genetic modifications in pigs. One such study involved the generation of *GGTA1*, *β2-microglobulin* (*β2M*), and *CIITA* triple knockout (GBC-3KO) pigs using CRISPR/Cas9 technology (Figure 3). This genetic modification effectively reduced hyperacute xenograft rejection and prolonged the survival of pig skin grafts in immunocompetent mice (Fu et al., 2020). Another experiment transplanted kidneys from genetically modified pigs into brain-dead human recipients. The kidneys began to produce urine almost immediately after reperfusion, and no signs of hyperacute or antibody-mediated rejection were observed over a 54-hour study period (Montgomery et al., 2022). These experiments highlight the potential of genetic modifications to mitigate immune responses and improve xenograft survival.

Fu et al. (2020) describes the creation of *GGTA1*−/−*β2M*−/−*CIITA*−/− triple gene knockout (GBC-3KO) pigs using CRISPR/Cas9 technology. Guide RNAs targeted exon 8 of *GGTA1*, exon 2 of *β2M*, and exon 9 of *CIITA*. The process involved transfecting pig embryonic fibroblasts (PEFs) with Cas9 and sgRNA vectors, followed by single-cell sorting and genotyping. Successful knockout cell lines underwent somatic cell nuclear transfer, leading to embryo implantation in surrogates. Of the 1 346 transferred embryos, five pregnancies resulted in two natural deliveries, producing five male piglets. Genotyping confirmed the knockout mutations. This research demonstrates effective genetic editing for creating multi-gene knockout pigs for potential xenotransplantation applications.

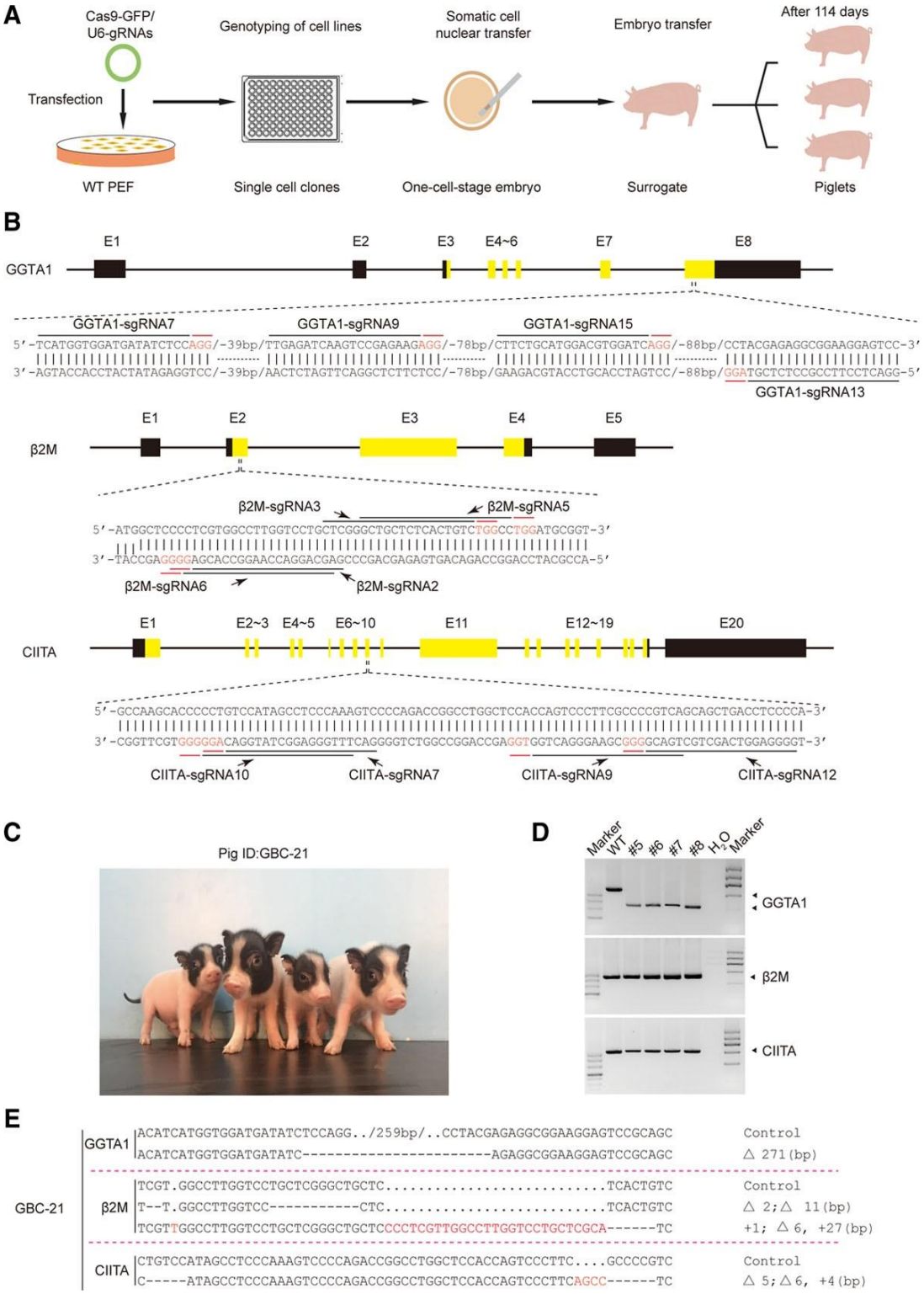

Figure 3 Generation of *GGTA1*−/−*β2M*−/−*CIITA*−/−triple gene knockout (GBC-3KO) pigs (Adopted from Fu et al., 2020)

Image caption: A, Schematic overview of the generation of GBC-3KO pigs. B, Illustration of the CRISPR/Cas9 targeting sites in *GGTA1*, *β2M*, and *CIITA* genes. The sgRNA targeting sequence is underlined in black, and the protospacer-adjacent motif (PAM) sequence is underlined and labeled in red. C, Photograph of GBC-3KO pigs from GBC-21 porcine embryonic fibroblast cell line. D, Genotyping of the GBC-3KO pigs by polymerase chain reaction (PCR). E, Genotyping of the GBC-3KO pigs from GBC-21 porcine embryonic fibroblast cell line by Sanger sequencing. The sizes of insertion (+) and deletion (Δ) are presented on the right side of each allele. *β2M*, β2-microglobulin; Cas9-eGFP, pUC19-pCAG-SpCas9-2A-GFP; *CIITA*, major histocompatibility complex class II transactivator; CRISPR/Cas9, a gene-editing technology; *GGTA1*, glycoprotein galactosyltransferase α 1, 3; sgRNA, single guide RNA sequence 7; PEF, pig embryonic fibroblast; U6-gRNA, pUC19-U6-sgRNA; WT, wild type (Adopted from Fu et al., 2020)

4.2 Long-term studies on survival and functionality of genetically modified pig organs

Long-term studies have shown promising results regarding the survival and functionality of genetically modified pig organs. Research has indicated that organs from pigs with multiple genetic modifications, such as the deletion of carbohydrate xenoantigens and the expression of human complement-regulatory proteins, can function for clinically valuable periods, exceeding 12 months in some cases (Cooper et al., 2019). Additionally, preclinical models have demonstrated prolonged xenograft survival times for various organs, including the heart, liver, kidney, and lung, in pig-to-non-human primate models (Lu et al., 2020). These findings suggest that genetically modified pig organs can maintain functionality and viability over extended periods, making them a viable option for clinical xenotransplantation.

4.3 Comparison of genetically modified organs versus non-modified controls

Comparative studies between genetically modified and non-modified pig organs have consistently shown superior outcomes for the former. For instance, GBC-3KO pig skin grafts exhibited significantly prolonged survival compared to wild-type pig skin grafts in immunocompetent mice (Fu et al., 2020). Similarly, kidneys from genetically modified pigs transplanted into brain-dead human recipients showed improved renal function and no signs of hyperacute rejection, whereas non-modified controls would likely have faced immediate rejection (Montgomery et al., 2022). These comparisons underscore the critical role of genetic modifications in enhancing the compatibility and performance of pig organs for xenotransplantation.

5 Ethical and Regulatory Considerations

5.1 Ethical issues surrounding genetic modifications in animals

The ethical implications of genetic modifications in animals, particularly pigs for xenotransplantation, are multifaceted and complex. One primary concern is the welfare of the genetically modified animals. These animals are often kept in laboratory conditions that may not meet their biological and psychological needs, raising significant animal welfare issues (Lei et al., 2022). The process of genetic modification itself, which includes techniques such as CRISPR/Cas9, can also be ethically contentious due to the potential for unforeseen consequences and the manipulation of animal genomes for human benefit (Kararoudi et al., 2018). Additionally, there are broader ethical debates about whether humans should engage in genetic engineering at all, with some arguing that it represents a form of technological overreach (Lei et al., 2022).

5.2 Regulatory frameworks governing xenotransplantation and genetic engineering

The regulatory landscape for xenotransplantation and genetic engineering is evolving but remains stringent. National regulatory authorities require extensive evidence to justify each genetic modification in donor pigs, often based on in vitro and in vivo experimental data (Cooper et al., 2019). The International Society for Heart and Lung Transplantation, for example, has set specific benchmarks for graft survival in large animal models before clinical trials can proceed (Mohiuddin et al., 2019). Regulatory frameworks also address the potential risks of zoonotic diseases, necessitating rigorous infectious disease surveillance and the notification of close contacts of recipients (Johnson, 2022). These frameworks aim to balance the potential benefits of xenotransplantation with the need to ensure safety and ethical integrity.

5.3 Public perception and ethical debates

Public perception of genetic modifications in animals and xenotransplantation is influenced by a variety of ethical and societal concerns. There is often a lack of understanding of the science behind these technologies, which can lead to spurious ethical concerns, such as the belief that xenotransplantation violates natural or religious principles (Rollin, 2020). Public debates also focus on the integrity and naturalness of animals, risk perception, and animal welfare issues (Eriksson et al., 2018). The ethical acceptability of using genetically modified animals for organ transplants is further complicated by concerns about justice and equity in organ distribution, as well as the potential exacerbation of existing healthcare inequities (Johnson, 2022). Engaging the public in informed discussions and addressing these ethical concerns transparently is crucial for the advancement of xenotransplantation technologies.

6 Technical Challenges and Limitations

6.1 Technical difficulties in gene editing and achieving stable modifications

Gene editing in pigs for xenotransplantation presents several technical challenges. One of the primary difficulties is achieving stable and precise genetic modifications. The use of CRISPR-Cas9 and other gene-editing technologies has significantly advanced the field, allowing for the deletion of specific pig genes and the insertion of human genes to reduce immunogenicity and improve compatibility (Sykes and Sachs, 2019). However, ensuring that these modifications are stable across generations and do not negatively impact the pigs' health or reproductive capabilities remains a significant hurdle (Yue et al., 2020). Additionally, the complexity of editing multiple genes simultaneously to address various immunological and physiological barriers adds another layer of difficulty (Deng et al., 2022; Lei et al., 2022).

6.2 Potential off-target effects and genetic instability

The potential for off-target effects is a major concern in gene editing. CRISPR-Cas9, while powerful, can introduce unintended mutations in the genome, which may lead to genetic instability or unforeseen health issues in the genetically modified pigs (Sykes and Sachs, 2019; Yue et al., 2020). These off-target effects can compromise the safety and efficacy of the xenotransplants. Moreover, the long-term stability of the genetic modifications is crucial, as any reversion or loss of the introduced traits could lead to graft rejection or other complications post-transplantation (Cooper et al., 2019). Continuous monitoring and advanced techniques to minimize off-target effects are essential to address these challenges.

6.3 Immunological challenges and unforeseen complications

Despite significant progress, immunological challenges remain a major barrier to successful xenotransplantation. Genetically modified pigs are designed to reduce hyperacute rejection, acute humoral xenograft rejection, and other immune responses (Deng et al., 2022; Montgomery et al., 2022). However, the human immune system is highly complex, and unforeseen complications can arise. For instance, while modifications such as the knockout of the alpha-1,3-galactosyltransferase gene have shown promise in reducing hyperacute rejection, other immune responses, such as chronic rejection and cell-mediated damage, still pose significant risks (Coe et al., 2020). Additionally, the risk of transmitting porcine endogenous retroviruses (PERVs) to human recipients remains a concern, despite efforts to inactivate these viruses in the pig genome (Yue et al., 2020). The interplay between the modified pig tissues and the human immune system needs further investigation to fully understand and mitigate these risks.

In conclusion, while genetic modifications in pigs offer a promising solution to the organ shortage crisis, several technical and immunological challenges must be addressed to ensure the safety and efficacy of xenotransplantation. Continuous advancements in gene-editing technologies and a deeper understanding of the immune responses involved are crucial for overcoming these barriers.

7 Future Directions and Perspectives

7.1 Emerging technologies and methods in genetic engineering

Recent advancements in genetic engineering have significantly enhanced the potential for creating immune-compatible organs from pigs for human transplantation. The development of CRISPR-Cas9 technology has been particularly transformative, allowing for precise and efficient genetic modifications. This technology has enabled the deletion of pig genes responsible for the synthesis of xenoantigens and the insertion of human genes that regulate immune responses and coagulation processes (Sykes and Sachs, 2019). Additionally, the use of transposon technologies in combination with CRISPR-Cas9 has facilitated extensive genome engineering, including the inactivation of porcine endogenous retroviruses (PERVs) and the introduction of multiple human transgenes to improve immunological compatibility (Yue et al., 2020). These emerging technologies are paving the way for more sophisticated and effective genetic modifications in pigs, which are crucial for the success of xenotransplantation.

7.2 Potential breakthroughs in immune-compatible organ engineering

The field of xenotransplantation is on the cusp of several potential breakthroughs that could revolutionize organ transplantation. One promising approach is the generation of humanized organs in pigs through interspecies blastocyst complementation. This method involves creating pig embryos deficient in specific developmental genes and complementing them with human induced pluripotent stem cells (hiPSCs) to generate organs with human endothelium, thereby reducing the risk of immune rejection (Das et al., 2020). Another significant breakthrough is the creation of genetically modified pigs with multiple gene edits, including the knockout of xenoantigens and the insertion of human transgenes. These modifications have shown promising results in preclinical studies, with some xenografts demonstrating long-term survival and function in non-human primates without signs of hyperacute rejection (Ma et al., 2020; Montgomery et al., 2022). These advancements suggest that genetically engineered pigs could soon provide a viable and sustainable source of organs for human transplantation.

7.3 Collaboration between researchers, clinicians, and policymakers

The successful translation of xenotransplantation from preclinical research to clinical practice will require close collaboration between researchers, clinicians, and policymakers. Researchers must continue to refine genetic engineering techniques and conduct rigorous preclinical studies to ensure the safety and efficacy of genetically modified pig organs (Li et al., 2021; Lei et al., 2022). Clinicians will play a critical role in designing and implementing clinical trials, as well as in managing the complex immunological challenges associated with xenotransplantation (Sykes and Sachs, 2019; Montgomery et al., 2022). Policymakers must establish clear regulatory frameworks to oversee the ethical and safe use of xenotransplantation in humans. This includes addressing concerns related to zoonotic disease transmission, long-term graft survival, and patient safety (Wolf et al., 2019; Xi et al., 2023). By fostering interdisciplinary collaboration and creating supportive regulatory environments, the potential of xenotransplantation to alleviate the organ shortage crisis can be fully realized.

In conclusion, the future of xenotransplantation looks promising, with emerging technologies and potential breakthroughs offering new hope for patients in need of organ transplants. Continued collaboration between all stakeholders will be essential to overcome the remaining challenges and bring this innovative solution to clinical reality.

8 Concluding Remarks

The advancements in genetic modifications of pigs for xenotransplantation have shown promising results in reducing immune rejection and improving the viability of pig organs in human recipients. Studies have demonstrated that genetically modified pig organs can function effectively in human recipients without signs of hyperacute rejection for extended periods. The use of CRISPR-Cas9 technology has enabled precise genetic modifications, such as the knockout of specific antigens and the insertion of human regulatory genes, which have significantly mitigated immune responses and physiological incompatibilities. These findings underscore the potential of xenotransplantation to address the critical shortage of human organs for transplantation.

The knockout of the alpha-1,3-galactosyltransferase gene and the insertion of human complement and coagulation regulatory genes have been pivotal in reducing hyperacute and acute rejection in xenotransplantation. This gene-editing tool has facilitated the creation of pigs with multiple genetic modifications, enhancing the compatibility of pig organs with the human immune system. Initial clinical trials have shown that genetically modified pig kidneys can function in human recipients for up to 54 hours without signs of rejection, indicating the feasibility of this approach. Understanding the immunological barriers and developing strategies to overcome them, such as the deletion of carbohydrate antigens and the expression of human complement regulatory proteins, have been crucial in advancing xenotransplantation.

Future research should focus on long-term studies to evaluate the durability and functionality of genetically modified pig organs in human recipients. Additionally, exploring the potential of interspecies chimeras and further refining genetic modifications to address remaining immunological challenges will be essential. Clinical applications will benefit from the development of standardized protocols for genetic modifications and the establishment of regulatory frameworks to ensure the safety and efficacy of xenotransplantation.

To realize the full potential of xenotransplantation, continued research and interdisciplinary collaboration are imperative. Researchers, clinicians, and regulatory bodies must work together to address the remaining challenges and translate these scientific advancements into clinical practice. Investment in research funding and the establishment of collaborative networks will be crucial in accelerating the development and implementation of xenotransplantation as a viable solution to the organ shortage crisis.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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