International Journal of Molecular Zoology 2024, Vol.14, No.4, 244-254
http://animalscipublisher.com/index.php/ijmz

Review and Progress Open Access

Pathogen-Free Pigs for Safer Xenotransplantation: Advances and Challenges in Genetic Engineering

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International Journal of Molecular Zoology, 2024, Vol.14, No.4 doi: 10.5376/ijmz.2024.14.0022
Received: 18 Jun., 2024
Accepted: 29 Jul., 2024
Published: 21 Aug., 2024

Preferred citation for this article:
Lin X.F., 2024, Pathogen-free pigs for safer xenotransplantation: advances and challenges in genetic engineering, International Journal of Molecular Zoology, 14(4): 244-254 (doi: 10.5376/ijmz.2024.14.0022)

Abstract Xenotransplantation, the transplantation of organs and tissues between different species, holds significant promise for addressing the shortage of human organs available for transplantation. Pigs are considered ideal donors due to their physiological similarities to humans. However, the risk of zoonotic pathogen transmission poses a substantial barrier to their use. This systematic review explores the latest advances in genetic engineering techniques aimed at creating pathogen-free pigs for safer xenotransplantation. We examine the current state of gene-editing technologies, such as CRISPR-Cas9, and their application in eliminating endogenous retroviruses and other pathogens. Additionally, we discuss the challenges faced in achieving complete pathogen eradication, including off-target effects, genetic stability, and ethical considerations. By evaluating the progress and limitations in this field, we aim to provide insights into the future directions for research and the potential impact of pathogen-free pigs on the safety and efficacy of xenotransplantation.

Keywords Xenotransplantation; Genetic engineering; Pathogen-free pigs; CRISPR-Cas9; Endogenous retroviruses

1 Introduction

Xenotransplantation, the process of transplanting organs, tissues, or cells from one species to another, has emerged as a promising solution to the critical shortage of human organs available for transplantation. The demand for organ transplants far exceeds the supply, with thousands of patients dying each year while waiting for a suitable donor organ (Wolf et al., 2019; Sykes and Sachs, 2019). Xenotransplantation, particularly using pigs as organ donors, has emerged as a promising solution to the organ shortage crisis (Lin, 2024). Pigs have been identified as the preferred donor species due to their anatomical and physiological similarities to humans, as well as their compatible organ sizes (Wolf et al., 2019; Ali et al., 2023). Recent advancements in genetic engineering have further enhanced the potential of pigs as viable organ donors by addressing key challenges such as immune rejection and physiological incompatibilities (Yue et al., 2020; Xi et al., 2023).

One of the major concerns in xenotransplantation is the risk of transmitting zoonotic pathogens from pigs to human recipients. Porcine endogenous retroviruses (PERVs) and other porcine viruses pose significant risks, as they can potentially infect human cells and compromise the safety of the transplant (Sykes and Sachs, 2019). To mitigate these risks, extensive efforts have been made to develop pathogen-free pigs through advanced genetic engineering techniques. For instance, the inactivation of PERVs using CRISPR-Cas9 technology has shown promising results in producing pigs that are free from these retroviruses (Yue et al., 2020). Additionally, the implementation of highly sensitive screening methods for detecting xenotransplantation-relevant viruses has further ensured the safety of using genetically modified pigs as organ donors (Denner, 2022).

This perspective paper aims to provide a comprehensive overview of the advances and challenges in the field of genetic engineering for creating pathogen-free pigs for safer xenotransplantation. By reviewing recent developments and highlighting key findings from various studies, this paper seeks to underscore the significance of genetic modifications in enhancing the safety and efficacy of pig-to-human organ transplants. By achieving these objectives, this paper aims to contribute to the ongoing efforts to make xenotransplantation a viable and safe alternative for patients in need of life-saving organ transplants. The insights provided herein will be valuable for

researchers, clinicians, and policymakers working towards the advancement of xenotransplantation technologies and the development of regulatory frameworks to ensure the safety and efficacy of these innovative medical procedures.

2 Background on Xenotransplantation

2.1 Definition and history of xenotransplantation

Xenotransplantation refers to the transplantation of living cells, tissues, or organs from one species to another. Historically, the concept of xenotransplantation has been explored as a potential solution to the shortage of human organs available for transplantation. Early attempts in the 20th century involved the use of primate organs, but these efforts were largely unsuccessful due to severe immune rejection and ethical concerns. The advent of genetic engineering has renewed interest in xenotransplantation, particularly using pigs as donors due to their anatomical and physiological similarities to humans (Wolf et al., 2019; Xi et al., 2023).

2.2 Current status of pig-to-human organ transplantation

Recent advancements in genetic engineering have made pigs a viable source of organs for human transplantation. Genetically modified pigs, specifically those with knockouts of the alpha-1,3-galactosyltransferase gene, have shown promise in reducing hyperacute rejection, a major barrier in xenotransplantation (Lei et al., 2022). Clinical studies have demonstrated that kidneys from these genetically modified pigs can function in human recipients for short periods without signs of hyperacute rejection (Montgomery et al., 2022). Additionally, ongoing research focuses on further genetic modifications to address other immune responses and physiological incompatibilities, paving the way for future clinical trials (Kemter et al., 2020).

2.3 Risks associated with pathogens in xenotransplantation

One of the significant risks in xenotransplantation is the potential transmission of zoonotic pathogens from donor pigs to human recipients. Pigs can harbor various infectious agents, including porcine endogenous retroviruses (PERVs), which pose a risk to immunocompromised transplant recipients (Hartline et al., 2018; Lei et al., 2022). To mitigate these risks, genetic modifications are being employed to knock down genes related to PERVs, and highly sensitive diagnostic methods are being developed to detect and monitor infectious agents in donor pigs and transplant recipients (Hartline et al., 2018). Ensuring the safety of xenotransplantation requires a comprehensive understanding of the spectrum of infectious agents in donor pigs and the implementation of stringent screening protocols.

3 Genetic Engineering Techniques

3.1 Overview of genetic engineering technologies

Genetic engineering technologies have revolutionized the field of xenotransplantation, particularly in the development of pathogen-free pigs. The primary tools used in genetic engineering include CRISPR/Cas9, TALENs (Transcription Activator-Like Effector Nucleases), and ZFNs (Zinc Finger Nucleases). CRISPR/Cas9: This technology allows for precise editing of the genome by creating double-strand breaks at specific locations, which are then repaired by the cell's natural repair mechanisms. CRISPR/Cas9 has been widely used due to its simplicity, efficiency, and versatility. It has been employed to inactivate porcine endogenous retroviruses (PERVs) and to modify genes that enhance immunological compatibility with humans (Kemter et al., 2018). TALENs: These are engineered proteins that can be designed to bind to specific DNA sequences and create double-strand breaks. TALENs offer high specificity and have been used in various genetic modifications, although they are generally more complex to design and produce compared to CRISPR/Cas9. ZFNs: These are synthetic proteins that can be engineered to target specific DNA sequences. ZFNs have been used for gene editing for many years, but their use has declined with the advent of more efficient technologies like CRISPR/Cas9.

3.2 Advantages and limitations of different gene editing tools

Each gene editing tool has its own set of advantages and limitations. The advantages of CRISPR/Cas9 are high efficiency, ease of design, and ability to target multiple genes simultaneously. However, when using CRISPR/Cas9, we also need to pay attention to potential off-target effects and the need for careful design to avoid unintended mutations (Kararoudi et al., 2018; Yue et al., 2020). TALENs has high specificity and lower off-target effects

compared to CRISPR/Cas9. But it is more complex and time-consuming to design and produce, which can limit their widespread use. ZFNs has long history of use and well-understood mechanisms, while has lower efficiency and higher cost compared to CRISPR/Cas9 and TALENs.

3.3 Specific applications of genetic engineering in creating pathogen-free pigs

Genetic engineering has been pivotal in creating pathogen-free pigs for safer xenotransplantation. Some specific applications include: Inactivation of PERVs: Using CRISPR/Cas9, researchers have successfully inactivated PERVs in the pig genome, reducing the risk of viral transmission during xenotransplantation (Kemter et al., 2018; Yue et al., 2020). Elimination of Xenoantigens: Genetic modifications have been made to eliminate specific carbohydrate antigens such as αGal, Neu5Gc, and Sd(a), which are targets for human antibodies. This reduces the risk of acute rejection of xenotransplants. Expression of Human Transgenes: Pigs have been engineered to express human complement regulatory proteins (e.g., hCD46, hCD55, hCD59) and other transgenes that enhance immunological compatibility and reduce coagulation-related issues during xenotransplantation (Yue et al., 2020). Improvement of Islet Function: Genetic modifications, such as the expression of glucagon-like peptide-1 and M3 muscarinic receptors, have been shown to increase insulin secretion in porcine islets, potentially improving the outcomes of islet xenotransplantation for diabetes treatment (Kemter et al., 2018).

In summary, the advancements in genetic engineering technologies, particularly CRISPR/Cas9, have significantly contributed to the development of pathogen-free pigs, enhancing the safety and efficacy of xenotransplantation. These technologies offer promising solutions to overcome immunological barriers and reduce the risk of pathogen transmission, paving the way for clinical applications in the near future.

4 Advances in Creating Pathogen-Free Pigs

4.1. Targeting endogenous retroviruses (e.g., PERVs) using gene editing

Porcine endogenous retroviruses (PERVs) are integrated into the genome of all pigs and pose a significant risk for xenotransplantation due to their potential to infect human cells. Recent advances in gene editing, particularly the use of CRISPR/Cas9, have enabled the inactivation of PERVs in pigs. This approach involves mutating the *pol* genes of PERVs to prevent their replication and transmission (Figure 1) (Denner, 2021). Studies have shown that PERV-inactivated pigs can be produced, significantly reducing the risk of PERV transmission during xenotransplantation (Kemter et al., 2018; Niu et al., 2020; Denner, 2022).

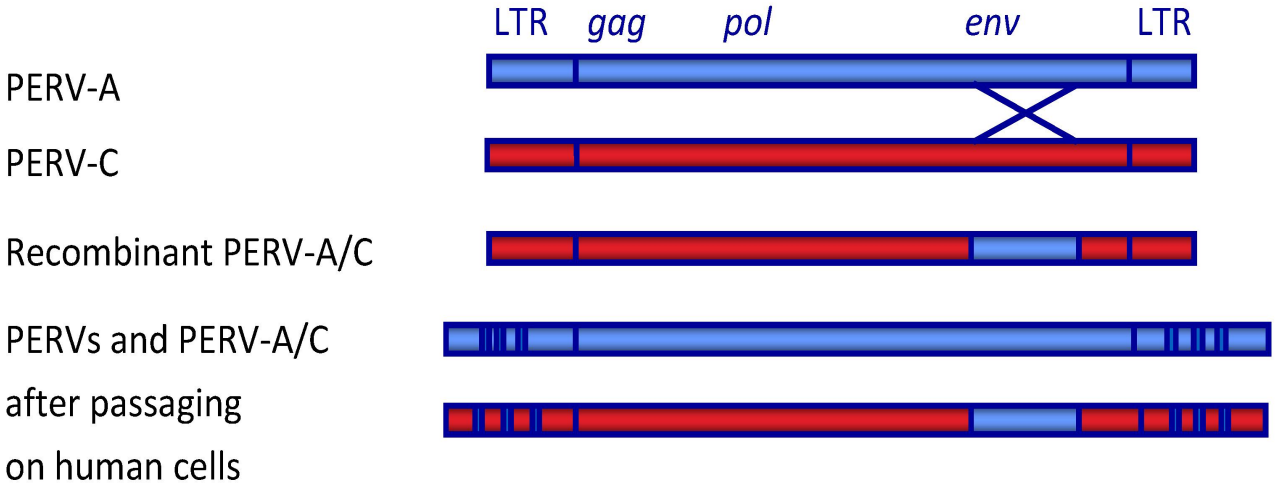

Figure 1 Schematic presentation of the genome of PERV (Adopted from Denner, 2021)

Image caption: LTR, long terminal repeat; *gag*, group specific antigen; *pol*, polymerase; *env*, envelope. The recombinant PERV-A/C is the result of a recombination in the *env* gene spanning the receptor binding domain (RBD). During passaging of PERV-A and PERV-A/C on human cells, a multimerization of repeats in the LTR takes place (Adopted from Denner, 2021)

The research of Denner (2021) presents a schematic representation of the genome structure of different types of Porcine Endogenous Retroviruses (PERVs), specifically PERV-A, PERV-C, and their recombinant form

PERV-A/C. PERV-A and PERV-C are shown with distinct color coding: blue for PERV-A and red for PERV-C. The recombinant PERV-A/C results from a recombination event in the env gene, where segments from both PERV-A and PERV-C are combined. Additionally, the schematic illustrates the structural changes occurring in PERVs and PERV-A/C genomes after being passaged on human cells. These changes include the multimerization of repeats in the long terminal repeat (LTR) regions. The detailed depiction of the *gag*, *pol*, and *env* genes along with the LTR highlights the genetic complexity and adaptability of these viral genomes under different conditions.

4.2 Strategies to eliminate other pathogens (e.g., bacteria, viruses, parasites)

In addition to targeting PERVs, various strategies have been developed to eliminate other pathogens from pigs used for xenotransplantation. These strategies include early weaning to prevent the transmission of porcine cytomegalovirus (PCMV) and porcine roseolovirus (PCMV/PRV), as well as the use of highly sensitive PCR-based and immunological methods for detecting and screening for numerous xenotransplantation-relevant viruses. These methods ensure that donor pigs are free from a wide range of pathogens, thereby enhancing the safety of xenotransplantation (Fishman, 2018; Denner, 2022; Lei et al., 2022).

4.3 Case studies and experimental results of pathogen elimination in pigs

Several case studies and experimental results have demonstrated the effectiveness of pathogen elimination strategies in pigs. For instance, in preclinical trials involving the transplantation of pig hearts into baboons, the transmission of PCMV/PRV was observed, which significantly reduced the survival time of the xenotransplant. However, early weaning was shown to eliminate PCMV/PRV from donor pigs. Additionally, no PERV transmission was observed in clinical trials involving the transplantation of pig islet cells into diabetic humans, indicating the success of pathogen elimination strategies in these cases (Denner, 2021; Eisenson et al., 2022).

4.4 Advances in genetic engineering for pathogen-free pigs

Genetic engineering has played a crucial role in creating pathogen-free pigs for xenotransplantation. By deleting genes related to the synthesis of pig-specific antigens and inserting human complement and coagulation-regulatory transgenes, researchers have been able to reduce the risk of immune rejection and physiological incompatibilities. Furthermore, the genetic modification of pigs to knock down genes related to PERVs has been a significant advancement in ensuring the safety of xenotransplantation. These genetic modifications, combined with technological breakthroughs in the biomedical field, provide a promising foundation for the future of pig-to-human xenotransplantation (Kemter et al., 2018; Niu et al., 2020).

5 Impact on Immunogenicity and Organ Compatibility

5.1 Effects of pathogen elimination on immunogenicity

The elimination of pathogens, particularly porcine endogenous retroviruses (PERVs), from genetically engineered pigs has shown significant promise in reducing immunogenicity. PERVs pose a risk of cross-species transmission, which can lead to immune responses in human recipients. Recent advancements using CRISPR-Cas9 technology have enabled the production of pigs with inactivated PERVs, thereby reducing the risk of zoonotic infections and subsequent immune reactions (Figure 2) (Denner, 2022). Additionally, the elimination of other porcine viruses, such as porcine cytomegalovirus (PCMV), has been shown to improve the survival time of xenotransplants by preventing virus-induced immune responses (Yue et al., 2020).

The research of Denner (2022) illustrates a process for inactivating Porcine Endogenous Retroviruses (PERVs) integrated into the pig genome using CRISPR/Cas technology. The process begins with embryonic fibroblasts containing integrated PERVs. CRISPR/Cas is applied to inactivate the PERVs by targeting and disabling the pol sequence. These modified fibroblasts are then used in somatic cell nuclear transfer (SCNT), where their nucleus is transferred into an oocyte (egg cell) that also contains the inactivated PERVs. The oocyte develops into an embryo, which is then implanted into a surrogate mother pig. The resulting piglets are born with inactivated PERVs in their genome, effectively preventing the potential transmission of these retroviruses. This method demonstrates a crucial step towards producing genetically modified pigs that are safer for xenotransplantation, reducing the risk of PERV transmission to humans.

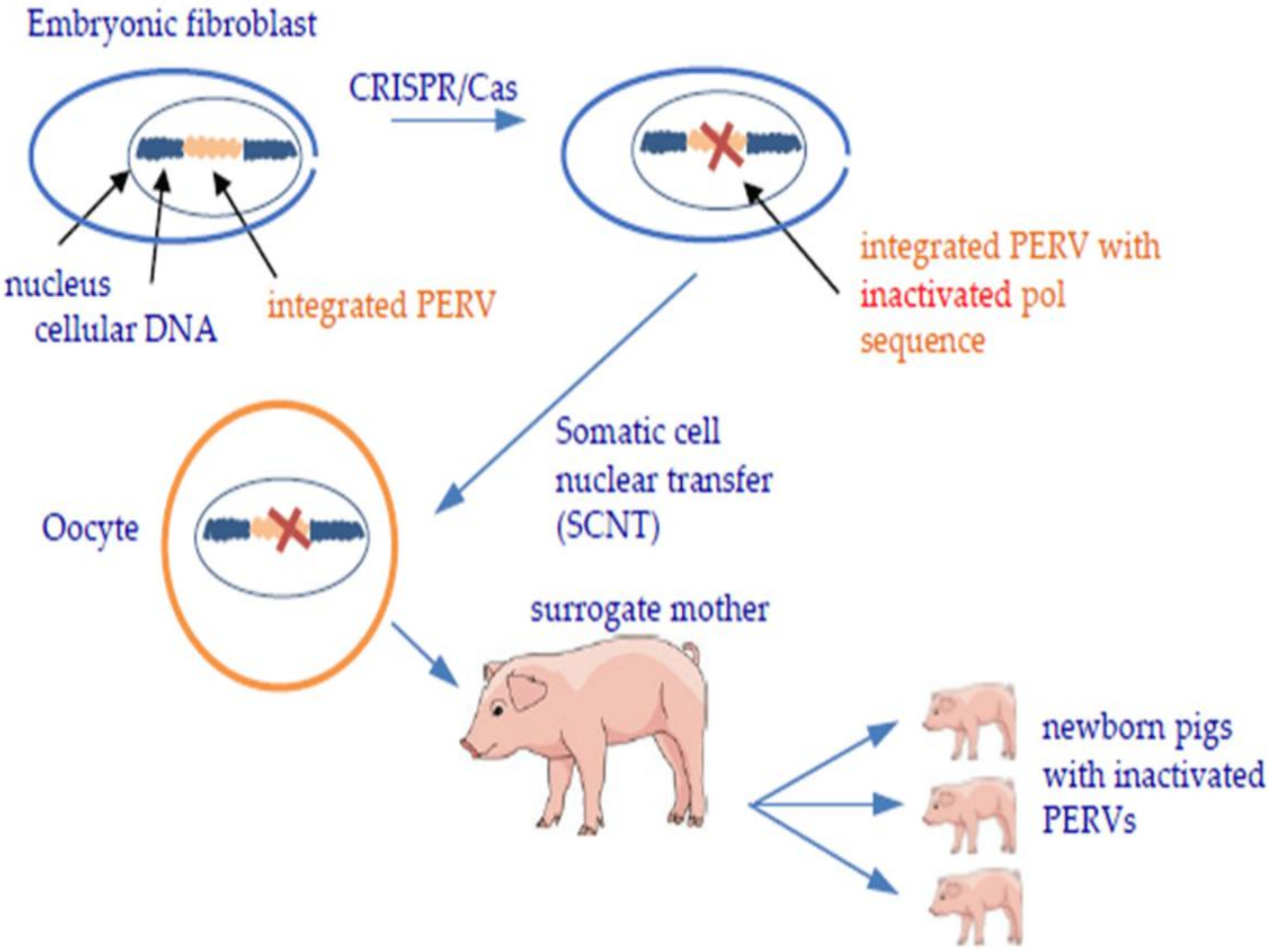

Figure 2 Inactivation of PERVs integrated in the pig genome using CRISPR/Cas and generation of piglets by somatic cell nuclear transfer (SCNT) (Adopted from Denner, 2022)

5.2 Enhancing organ compatibility through genetic modifications

Genetic modifications in pigs have been pivotal in enhancing organ compatibility for xenotransplantation. By deleting pig genes responsible for the synthesis of xenoantigens and introducing human transgenes, researchers have been able to reduce immune rejection and improve physiological compatibility. For instance, the deletion of genes encoding for carbohydrate antigens such as αGal, Neu5Gc, and Sd(a) has been shown to prevent the activation of human natural antibodies and complement systems (Kemter et al., 2018). Furthermore, the expression of human complement regulatory proteins (e.g., hCD46, hCD55, hCD59) in genetically modified pigs has been effective in mitigating cellular immune responses and improving graft survival (Figure 3) (Lei et al., 2022). These genetic modifications have extended the survival of pig organs in non-human primates, paving the way for potential clinical applications (Kemter et al., 2020; Wu et al., 2023).

The research of Lei et al. (2022) provides an overview of the mechanisms behind antibody-mediated xenograft rejection and the genetic modifications used to mitigate these immune responses. In hyperacute rejection (HAR), preformed antibodies bind to α-Gal antigens on pig endothelial cells (pEC), triggering the classical complement cascade that culminates in cell lysis and graft damage. To combat this, genetically modified pigs are produced with knockouts in genes such as *GGTA1*, *CMAH*, and *β4GalNT2*, which eliminate key xenoantigens. Additionally, transgenic pigs express human complement regulatory proteins (hCD55, hCD46, hCD59) to inactivate components of the complement cascade, thereby reducing cytolysis and thrombosis. Acute humoral xenograft rejection (AHXR) is also addressed by these genetic modifications, targeting antibodies against non-α-Gal antigens like Neu5Gc and SDa. This comprehensive approach enhances the compatibility of pig organs for transplantation into humans, improving graft survival and function.

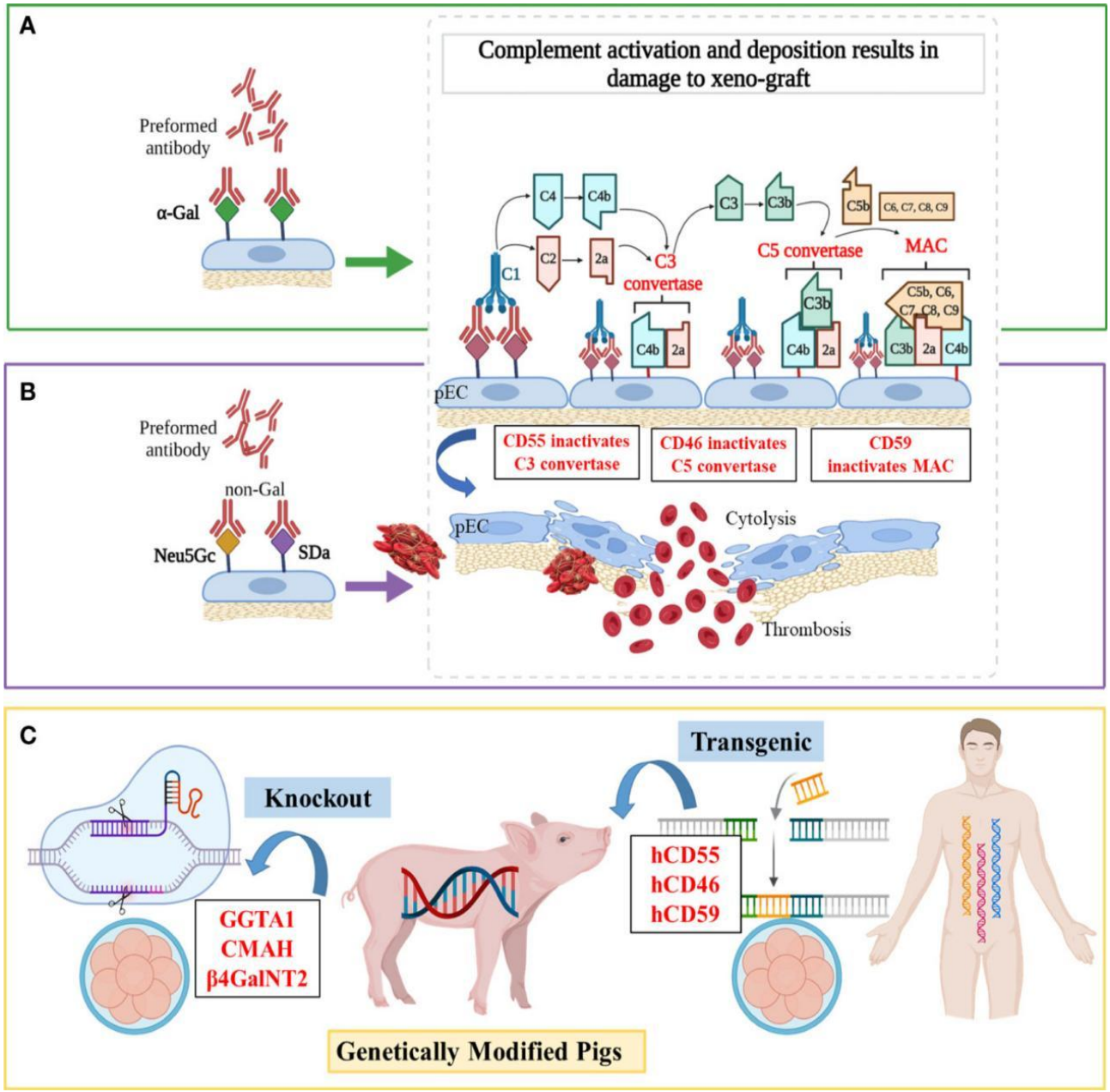

Figure 3 Mechanisms of antibody-mediated xenograft rejection and related genetic targets (Adopted from Lei et al., 2022)

Image caption: (A) Hyperacute rejection (HAR). In the classical complement activation cascade, the Fc regions of antibodies contact C1q, causing C1r and then C1s to autoactivate. Afterward, C4 and C2 are cleaved by activated C1s, resulting in the production of C4b2a (also known as C3 convertase). C3 convertase then cleaves C3 into C3a and C3b, with C3b binding to C4b2a to generate C4b2a3b (also known as C5 convertase). C5 convertase cleaves C5 into C5a and C5b, and then C5b is attached to C6, C7, C8, and multiple molecules of C9 to create the membrane attack complex (MAC), which ultimately causes cytolysis. (B) Acute humoral xenograft rejection (AHXR). AHXR can be induced by low levels of natural and elicited xenoreactive antibodies directed at non-α-Gal antigens (predominantly anti-Neu5Gc and -SDa), which also lead to complement activation via the classical pathway. The histopathology of AHXR is characterized by progressive destruction of the microvasculature (glomeruli and peritubular capillaries) and formation of fibrin-platelet thrombi. (C) Gene modifications. To prevent HAR, *CD59*, *CD55*, *hCD46* and *GGTA1* were modified. To shun AHXR, *CMAH*$^{-/-}$ and *β4GalNT2*$^{-/-}$ knockout pigs were produced. Notably, these target genes were not edited individually, but in combination to obtained better tolerized pig xenograft (Adopted from Lei et al., 2022)

5.3 Case studies of successful pathogen-free pig organ transplants

Several preclinical studies have demonstrated the success of pathogen-free pig organ transplants. For example, genetically engineered pigs with multiple modifications, including PERV inactivation and the expression of human transgenes, have shown normal physiology and fertility, with their organs exhibiting resistance to human

immune rejection in vitro (Yue et al., 2020). In another study, the transplantation of pig hearts into baboons, with the elimination of PCMV, resulted in significantly improved survival times, highlighting the importance of pathogen elimination in successful xenotransplantation (Denner, 2022). These case studies underscore the potential of genetically modified, pathogen-free pigs as viable organ donors for human transplantation, offering a promising solution to the organ shortage crisis (Wolf et al., 2019; Niu et al., 2020).

By addressing both immunogenicity and organ compatibility through advanced genetic engineering techniques, the field of xenotransplantation is making significant strides towards safer and more effective clinical applications.

6 Ethical and Regulatory Considerations

6.1 Ethical issues in the genetic engineering of animals

The genetic engineering of pigs for xenotransplantation raises several ethical concerns. One primary issue is the welfare of the genetically modified animals. The process of genetic modification and the subsequent use of these animals for organ harvesting can lead to significant suffering and raises questions about the moral status of these animals (Kemter et al., 2020). Additionally, there are concerns about the long-term ecological impacts of releasing genetically modified organisms into the environment, even if unintentionally (Wolf et al., 2019). The potential for creating interspecies chimeras, which involves combining human and animal cells, further complicates the ethical landscape, as it challenges traditional boundaries between species and raises questions about the nature of human identity and dignity (Xi et al., 2023).

6.2 Regulatory frameworks for approving genetically engineered pigs for xenotransplantation

The regulatory landscape for genetically engineered pigs intended for xenotransplantation is complex and varies significantly across different jurisdictions. In the United States, the Food and Drug Administration (FDA) oversees the regulation of genetically modified animals under the New Animal Drug Application (NADA) process, which requires extensive safety and efficacy data before approval (Denner, 2022). The European Medicines Agency (EMA) has similar stringent requirements, focusing on the safety of both the recipient and the broader public health implications (Sykes and Sachs, 2019). These regulatory frameworks are designed to ensure that genetically engineered pigs do not pose undue risks, particularly concerning the transmission of zoonotic diseases and the long-term health of transplant recipients.

6.3 Public perception and acceptance of genetically modified pigs

Public perception and acceptance of genetically modified pigs for xenotransplantation are critical factors that can influence the success of this technology. Public concerns often revolve around the safety and ethical implications of using genetically modified organisms. There is a significant need for transparent communication and public engagement to address these concerns and build trust. Studies have shown that public acceptance can be influenced by the perceived benefits of the technology, such as alleviating the organ shortage crisis, and by assurances regarding the safety and ethical treatment of the animals involved (Kemter et al., 2020; Lei et al., 2022). Effective regulatory oversight and clear ethical guidelines can also play a crucial role in shaping public opinion and acceptance (Sykes and Sachs, 2019; Denner, 2022).

7 Challenges and Limitations

7.1 Technical challenges in achieving complete pathogen elimination

Achieving complete pathogen elimination in genetically engineered pigs for xenotransplantation is a significant technical challenge. Despite the establishment of Designated Pathogen-Free (DPF) facilities and rigorous screening methods, the risk of pathogen transmission remains. For instance, porcine endogenous retroviruses (PERVs) are integrated into the pig genome and cannot be eliminated by conventional methods, posing a potential risk for zoonotic transmission (Denner, 2022). Additionally, environmental factors and human activities can lead to outbreaks of pathogens such as porcine circovirus (PCV), even in controlled settings (Noordergraaf et al., 2018). Advanced genome editing techniques like CRISPR-Cas9 have been employed to inactivate PERVs, but the complete elimination of all potential pathogens remains a complex and ongoing challenge (Yue et al., 2020).

7.2 Potential off-target effects and genetic stability

The use of CRISPR-Cas9 and other gene-editing technologies to create genetically modified pigs introduces the risk of off-target effects, which can lead to unintended genetic alterations. These off-target effects can compromise the genetic stability of the modified pigs, potentially leading to unforeseen health issues or reduced viability of the xenografts (Sykes and Sachs, 2019). Ensuring the precision and accuracy of genetic modifications is crucial, as even minor off-target effects can have significant implications for the safety and efficacy of xenotransplantation (Kemter et al., 2020). Continuous monitoring and validation of the genetic modifications are necessary to mitigate these risks and ensure the long-term stability of the engineered pigs (Yue et al., 2020).

7.3 Long-term health and viability of genetically modified pigs

The long-term health and viability of genetically modified pigs are critical factors for the success of xenotransplantation. While initial studies have shown that genetically engineered pigs can exhibit normal physiology, fertility, and germline transmission of edited genes, the long-term effects of extensive genetic modifications are still not fully understood (Yue et al., 2020; Lei et al., 2022). There is a need for comprehensive longitudinal studies to assess the health, reproductive capabilities, and overall viability of these pigs over their lifespan. Additionally, the potential impact of genetic modifications on the pigs' immune system and their ability to resist infections must be thoroughly investigated to ensure the sustainability of xenotransplantation efforts (Wolf et al., 2019).

8 Future Directions and Perspectives

8.1 Emerging trends and innovations in genetic engineering for xenotransplantation

Recent advancements in genetic engineering have significantly enhanced the potential of xenotransplantation. The use of CRISPR-Cas9 and transposon technologies has enabled the production of pigs with inactivated porcine endogenous retroviruses (PERVs) and the introduction of human transgenes to improve immunological compatibility and blood-coagulation compatibility with humans (Yue et al., 2020). Additionally, the development of pigs with multiple genetic modifications, such as the deletion of pig-specific antigens and the insertion of human complement and coagulation-regulatory transgenes, has shown promise in overcoming immune rejection and physiological incompatibilities (Wolf et al., 2019; Lei et al., 2022). These innovations are paving the way for safer and more effective xenotransplantation practices.

8.2 Potential breakthroughs and future vision for pathogen-free pigs

The creation of pathogen-free pigs is a critical goal for the future of xenotransplantation. Advances in genome editing have allowed for the inactivation of PERVs, which are integrated into the pig genome and pose a risk of cross-species transmission (Denner, 2022). Furthermore, the development of highly sensitive diagnostic methods for detecting xenotransplantation-relevant viruses, such as porcine cytomegalovirus (PCMV) and porcine circovirus 3 (PCV3), is essential for ensuring the safety of xenotransplant recipients (Hartline et al., 2018). Future research should focus on refining these techniques and exploring additional genetic modifications to eliminate other potential zoonotic pathogens, thereby creating a safer and more reliable source of organs for transplantation (Karuppannan and Opriessnig, 2018).

8.3 Collaborative and interdisciplinary research opportunities

The complexity of xenotransplantation necessitates a collaborative and interdisciplinary approach. Researchers from fields such as genetics, immunology, virology, and bioethics must work together to address the various challenges associated with xenotransplantation. For instance, the ethical considerations of using genetically modified pigs for organ transplantation require input from bioethicists to ensure that the benefits outweigh the potential harms (Cengiz and Wareham, 2019). Additionally, collaboration between virologists and genetic engineers is crucial for developing strategies to eliminate or mitigate the risks of cross-species virus transmission (Karuppannan and Opriessnig, 2018; Denner, 2022). By fostering interdisciplinary research, the scientific community can accelerate the development of safe and effective xenotransplantation practices.

9 Concluding Remarks

The research on genetically engineered pigs for xenotransplantation has made significant strides in addressing the critical shortage of human donor organs. Key advancements include the development of pigs with inactivated porcine endogenous retroviruses (PERVs) and multiple genetic modifications to enhance immunological compatibility and reduce the risk of zoonotic infections. These modifications include the deletion of pig-specific antigens and the insertion of human complement and coagulation-regulatory transgenes, which have shown promising results in preclinical studies. Additionally, the establishment of designated pathogen-free (DPF) pig herds has been crucial in minimizing the risk of virus transmission during xenotransplantation.

The progress in genetic engineering of pigs opens new avenues for clinical applications of xenotransplantation. Future research should focus on optimizing the genetic modifications to further reduce immunological barriers and enhance the longevity and functionality of xenografts in human recipients. Moreover, the development of more sophisticated screening and monitoring techniques for potential zoonotic pathogens, including single-stranded DNA viruses, is essential to ensure the safety of xenotransplantation procedures. Clinical trials are needed to validate the efficacy and safety of these genetically engineered pigs in human patients, which will require rigorous regulatory oversight and ethical considerations.

Continued research is imperative to address the remaining challenges in xenotransplantation, including the refinement of genetic modifications and the prevention of cross-species virus transmission. Researchers and clinicians must collaborate to develop standardized protocols for the production and screening of pathogen-free pigs. Ethical considerations, such as the potential psychological impact on recipients and the broader societal implications of xenotransplantation, must be carefully evaluated and addressed. It is crucial to engage with regulatory bodies, ethicists, and the public to ensure that the benefits of xenotransplantation are realized in a responsible and ethically sound manner.

In conclusion, while significant progress has been made in the field of xenotransplantation, ongoing research and ethical vigilance are essential to overcome the remaining hurdles and to bring this promising solution to clinical reality.

Acknowledgments

Author extends sincere thanks to two anonymous peer reviewers for their invaluable feedback on the initial draft of this manuscript.

Conflict of Interest Disclosure

Author affirms 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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