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International Journal of Clinical Case Reports 2024, Vol.14, No.2, 91-103
http://medscipublisher.com/index.php/ijccr

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Gene Editing for Organ Transplants: Evaluating the Impact of CRISPR/Cas9 on Immunogenicity and Organ Longevity in Pigs

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International Journal of Clinical Case Reports 2024, Vol.14, No.2 doi: 10.5376/ijccr.2024.14.0012
Received: 28 Mar., 2024
Accepted: 30 Apr., 2024
Published: 10 May., 2024

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Preferred citation for this article:
Li J.H., and Li J., 2024, Gene editing for organ transplants: evaluating the impact of CRISPR/Cas9 on immunogenicity and organ longevity in pigs, International Journal of Clinical Case Reports, 14(2): 91-103 (doi: 10.5376/ijccr.2024.14.0012)

Abstract This study explores the application of CRISPR-Cas9 gene editing technology in organ transplantation, particularly its potential in reducing immunogenicity and enhancing the longevity of pig organs. The research focuses on strategies to reduce immune rejection through gene editing, specifically targeting genes such as *GGTA1*, *CMAH,* and *β4GalNT2*. Experimental results demonstrate that these genetic modifications significantly reduce the immunogenicity of pig organs. The study also investigates mechanisms to improve organ tolerance and function through gene editing, such as upregulation of anti-apoptotic and antioxidant genes, and strategies to mitigate ischemia-reperfusion injury. The article concludes with an assessment of the potential breakthroughs and future prospects of CRISPR-Cas9 in xenotransplantation, emphasizing the technical challenges and ethical considerations that need to be addressed in future research and clinical applications.

Keywords CRISPR-Cas9; Gene editing; Immunogenicity; Xenotransplantation

Organ transplantation remains the definitive treatment for end-stage organ failure, significantly enhancing both the quality and longevity of life for patients with terminal heart, lung, liver, and kidney diseases. Despite advancements, the availability of suitable donor organs falls critically short of demand. This shortage results in high mortality rates among patients on transplant waiting lists, with over 10-15% of heart and liver transplant candidates dying each year while awaiting a compatible donor (Cravedi, 2019). This gap between supply and demand has prompted the exploration of alternative sources of organs, including xenotransplantation, which involves the transplantation of animal organs into humans. Pigs are particularly promising donors due to their physiological and anatomical similarities to humans, as well as their potential for genetic modification to reduce immunological barriers (Ekser et al., 2015).

Gene editing, especially with the advent of CRISPR-Cas9 technology, has revolutionized the field of genetic engineering. CRISPR-Cas9 allows for precise modifications of the genome by targeting specific DNA sequences and introducing changes such as deletions, insertions, or replacements. This technology has been instrumental in creating genetically modified pigs that are better suited for xenotransplantation. For example, CRISPR-Cas9 has been used to inactivate porcine endogenous retroviruses (PERVs), which are integrated into the pig genome and pose a risk of cross-species viral transmission during xenotransplantation (Niu et al., 2017). These advancements have significantly improved the safety and viability of using pig organs for human transplants.

The primary objective of this research is to evaluate the impact of CRISPR-Cas9-mediated gene editing on the immunogenicity and longevity of pig organs used in xenotransplantation. Immunogenicity refers to the ability of the transplanted organ to elicit an immune response in the human recipient, which can lead to rejection of the organ. By using CRISPR-Cas9 to modify specific genes, it is possible to reduce or eliminate these immune responses, thereby increasing the success rates of xenotransplantation. Additionally, understanding how these genetic modifications affect organ longevity is crucial for ensuring that the transplanted organs function effectively over long periods. This research holds significant potential to address the critical shortage of human

donor organs, ultimately saving countless lives by providing a reliable alternative through xenotransplantation with genetically modified pigs (Hryhorowicz et al., 2017; Wolf et al., 2019).

1 Background on CRISPR-Cas9 Technology

1.1 Mechanism and principles of CRISPR-Cas9

CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9) is a revolutionary genome-editing tool derived from the adaptive immune system of bacteria and archaea. This system provides these microorganisms with a defense mechanism against invading viruses and plasmids by targeting and cleaving their DNA.

The mechanism of CRISPR-Cas9 involves two key components: the Cas9 protein and a guide RNA (gRNA). The gRNA is composed of two parts: a CRISPR RNA (crRNA) that recognizes the target DNA sequence and a trans-activating crRNA (tracrRNA) that binds to the Cas9 protein. Together, they form a complex that scans the genome to find and bind to a specific DNA sequence complementary to the crRNA. Once bound, the Cas9 protein induces a double-strand break at the target site. This break is then repaired by the cell's natural repair mechanisms, either through non-homologous end joining (NHEJ) or homology-directed repair (HDR), allowing for the introduction of specific genetic changes (Wang et al., 2016).

1.2 Advantages of CRISPR-Cas9 over traditional gene editing techniques

CRISPR-Cas9 offers several advantages over traditional gene editing techniques such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). CRISPR-Cas9 requires only a single guide RNA to direct the Cas9 protein to the target DNA, whereas ZFNs and TALENs require the engineering of specific proteins for each target sequence. This makes CRISPR-Cas9 simpler and more cost-effective (Sun et al., 2017).

CRISPR-Cas9 can target multiple genes simultaneously by using different gRNAs, allowing for the editing of several genes in a single experiment. This is particularly useful for studies involving complex genetic interactions (Lowder et al., 2015). The CRISPR-Cas9 system can be easily adapted for a variety of applications beyond simple gene editing, including gene activation (CRISPRa), gene repression (CRISPRi), epigenetic modifications, and live-cell imaging of chromosomal loci (Wang and Qi, 2016). High Precision: The ability to design specific guide RNAs for precise targeting of DNA sequences allows CRISPR-Cas9 to introduce genetic modifications with high accuracy, reducing off-target effects compared to other methods (Mollanoori and Teimourian, 2018).

1.3 Applications of CRISPR-Cas9 in biomedical research

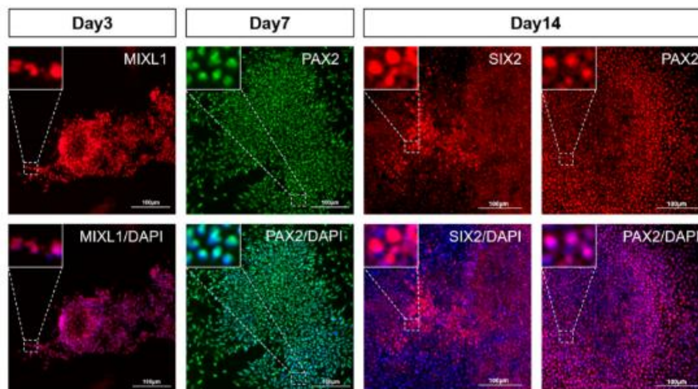
CRISPR-Cas9 has wide-ranging applications in biomedical research, demonstrating its versatility and transformative impact. CRISPR-Cas9 has been used to create precise genetic models of human diseases in animals. For example, it has facilitated the development of rodent models with specific mutations to study cancer, neurodegenerative diseases, and metabolic disorders (Collins et al., 2017). CRISPR-Cas9 holds promise for therapeutic gene editing to correct genetic defects. Clinical trials are underway to evaluate its efficacy in treating conditions such as sickle cell anemia, beta-thalassemia, and certain types of cancer by editing patient-derived cells (Li et al., 2021).

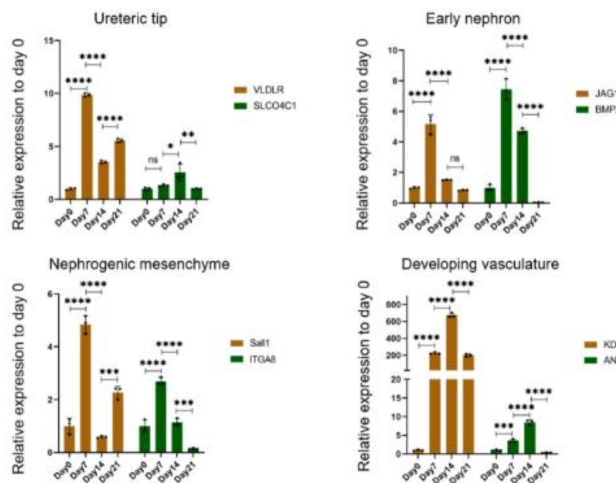
By enabling the systematic disruption of genes, CRISPR-Cas9 has accelerated functional genomics studies, helping to identify genes essential for various biological processes and disease states (Ratan et al., 2018). Beyond medical applications, CRISPR-Cas9 is also revolutionizing agricultural biotechnology by enhancing crop resistance to diseases and environmental stresses, improving yield, and modifying nutritional content (Liu et al., 2017). CRISPR-Cas9 is used in drug discovery and development to identify potential drug targets and understand drug interactions at the genetic level. This helps in the creation of more effective and targeted therapies (He et al., 2022).

2 Pigs as Organ Donors

2.1 Rationale for using pigs in xenotransplantation

Pigs have emerged as the most suitable donor species for xenotransplantation due to several compelling reasons. Their organs are similar in size and physiology to human organs, making them an ideal match for transplantation needs. Additionally, pigs have a relatively short gestation period and large litters, allowing for the efficient production of donor animals. The ability to raise pigs in specific pathogen-free environments further minimizes the risk of zoonotic diseases (Hryhorowicz et al., 2017; Wolf et al., 2019). Importantly, advances in genetic engineering have made it possible to modify pigs genetically to reduce immunogenicity and enhance compatibility with human recipients (Ekser et al., 2009) (Figure 1).





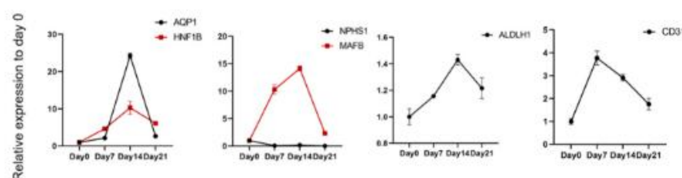


Figure 1 Changes in gene expression in 2D cultured porcine kidney organoids (Adopted from Li et al., 2018)

Image caption: (A) Expression of special markers in different periods. The specific markers for PS cells (MIXL1), IM cells (PAX2), MM cells (SIX2 and PAX2), and UE cells (SIX2 and PAX2) were discovered in the corresponding porcine kidney organoids on days 3, 7, and 14 of 2D culture by immunofluorescence test (MIXL1, PAX2, and SIX2 were all localized to the nucleus) (scale bar: 100 μm). (B) Expression level changes in renal progenitor cell markers at 4 time points (day 0, 7, 14, and 21) of the 2D cultured porcine kidney organoids. The relative expression levels of markers about early nephrons (JAG1, BMP2), nephrogenic stroma (SALL1, ITGA8), ureteral buds (VLDLR, SLCO4C1), and endothelial progenitor cells (KDR, ANGPT2) were measured by real-time PCR. (C) Expression level changes in mature nephron components markers at 4 time points (day 0, 7, 14, and 21) of the 2D cultured porcine kidney organoids (Adopted from Li et al., 2018).

2.2 Challenges in pig-to-human organ transplants

While the use of pigs as organ donors offers significant potential, it also presents several challenges that must be addressed for successful xenotransplantation. The immune system's response to foreign tissues is a major barrier. Pigs express the alpha-1,3-galactosyltransferase (α-Gal) antigen, which triggers hyperacute rejection (HAR) in humans. Genetic modifications, such as the knockout of the α-Gal gene and the introduction of human complement regulatory proteins, have been developed to mitigate this issue (Klymiuk et al., 2010; Gock et al., 2011).

Physiological differences between pigs and humans can lead to issues with organ functionality and longevity. For instance, differences in coagulation pathways can cause thrombotic microangiopathy in transplanted organs. Genetic engineering approaches aim to address these incompatibilities by incorporating human genes that regulate coagulation and immune responses (Lei et al., 2022; Wu et al., 2023). The potential transmission of porcine endogenous retroviruses (PERVs) to human recipients is a concern. Recent advances have made it possible to inactivate these viruses using CRISPR-Cas9 technology, reducing the risk of cross-species infections (Wolf et al., 2019).

2.3 Current status and advancements in pig organ transplantation

The field of xenotransplantation has made significant strides in recent years, bringing the prospect of clinical application closer to reality. The creation of genetically modified pigs that lack α-Gal and express human complement regulatory proteins has greatly improved the survival rates of pig organs in non-human primate models. For instance, hearts and kidneys from genetically modified pigs have survived for several months in primates, demonstrating the potential for longer-term graft function (Ekser et al., 2009; Hryhorowicz et al., 2017; Längin et al., 2018) (Figure 2).

Advances in immunosuppressive therapies have been crucial in extending the viability of xenografts. The development of novel immunosuppressive agents that target specific immune pathways has helped reduce the incidence of acute humoral xenograft rejection (AHXR) and other immune-mediated complications (Ekser et al., 2009; Zhang et al., 2020). Ongoing preclinical trials and regulatory discussions are paving the way for the first clinical trials of pig organ xenotransplantation. These trials will be critical in assessing the safety, efficacy, and long-term outcomes of xenotransplantation in human patients (Wolf et al., 2019; Ali et al., 2023).

3 CRISPR-Cas9 and Immunogenicity

3.1. Strategies for reducing immunogenicity through gene editing

CRISPR-Cas9 has revolutionized the field of xenotransplantation by enabling precise genetic modifications to reduce the immunogenicity of pig organs. The primary strategy involves knocking out genes that encode for xenoantigens responsible for triggering immune responses in human recipients. The main antigens targeted include galactose-α1,3-galactose (α-Gal), N-glycolylneuraminic acid (Neu5Gc), and Sd(a) antigen. By using CRISPR-Cas9 to disrupt the genes responsible for these antigens (GGTA1, CMAH, and β4GalNT2), researchers have significantly reduced the immunogenicity of pig tissues (Wang et al., 2018; Yoon et al., 2022).

Additionally, further strategies involve creating multi-gene knockouts to eliminate other immune targets such as the major histocompatibility complex (MHC) antigens SLA-I and SLA-II. This approach involves the simultaneous deletion of multiple genes, which can be efficiently achieved using CRISPR-Cas9 (Fu et al., 2020).

3.2 Specific genes targeted to minimize immune rejection

This gene encodes α1,3-galactosyltransferase, which is responsible for the synthesis of the α-Gal antigen. Knockout of GGTA1 has been shown to significantly reduce hyperacute rejection in xenotransplantation (Tanihara et al., 2021). This gene encodes CMP-N-acetylneuraminic acid hydroxylase, which synthesizes Neu5Gc, another xenoantigen. Combined knockout of GGTA1 and CMAH has been shown to further decrease the immune response compared to GGTA1 knockout alone (Gao et al., 2016).

This gene encodes β-1,4-N-acetyl-galactosaminyl transferase 2, responsible for the synthesis of Sd(a) antigen. Triple knockout of GGTA1, CMAH, and β4GalNT2 results in even lower human antibody binding and reduced immunogenicity (Zhang et al., 2018). These genes encode the swine leukocyte antigens, which are the porcine equivalents of human MHC molecules. Their knockout can reduce cellular immune responses against pig organs (Fu et al., 2020).

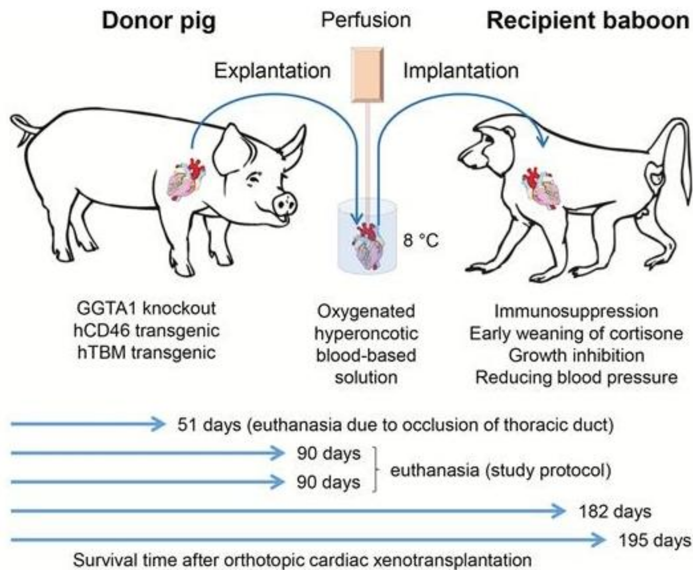


Figure 2 Factors enabling consistent success in life-supporting pig-to-baboon cardiac xenotransplantation (Adopted from Längin et al., 2018)

Image caption: In addition to genetically multimodified porcine donor hearts (lacking αGal epitopes and expressing human CD46 as well as human thrombomodulin) and appropriate immunosuppression, two steps were key to success: 1) nonischemic preservation of the donor hearts by perfusion with oxygenated hyperoncotic blood-based solution; and 2) prevention of detrimental xeno-heart overgrowth by early weaning of cortisone, lowering of blood pressure and treatment with the mTOR inhibiting prodrug temsirolimus (Adopted from Längin et al., 2018)

3.3 Case studies and experimental results on immunogenicity reduction

Triple Gene Knockout Pigs Research has shown that pigs with knockouts in *GGTA1*, *CMAH*, and *β4GalNT2* exhibit significantly reduced levels of human IgG and IgM binding. In a study by Wang et al. (2018), tissues from these triple knockout (TKO) pigs demonstrated minimal human antibody binding, indicating a substantial reduction in immunogenicity. These TKO pigs were produced using CRISPR-Cas9, which successfully eliminated the expression of α-Gal, Neu5Gc, and Sd(a) antigens.

Another study by Yoon et al. (2022) developed triple knockout JNPs using CRISPR-Cas9, targeting GGTA1, CMAH, and B4GALNT2. These pigs showed no expression of the three antigens in major organs, and human IgM and IgG binding was significantly reduced compared to wild-type pigs, supporting the potential use of these organs in xenotransplantation (Figure 3). Zhang et al. (2018) investigated the use of TKO pigs for BHVs. Their findings demonstrated that valves from TKO pigs exhibited reduced human IgM/IgG binding and had similar collagen composition and physical properties compared to wild-type pigs, making them a promising alternative for reducing immunogenicity in heart valve replacements. Fu et al. (2020) generated pigs with triple knockout of *GGTA1*, *β2M*, and *CIITA* genes, reducing both humoral and cellular immune responses. These pigs showed prolonged survival of skin grafts in immunocompetent mice, demonstrating the effectiveness of multi-gene knockouts in reducing xenogeneic immune reactions.

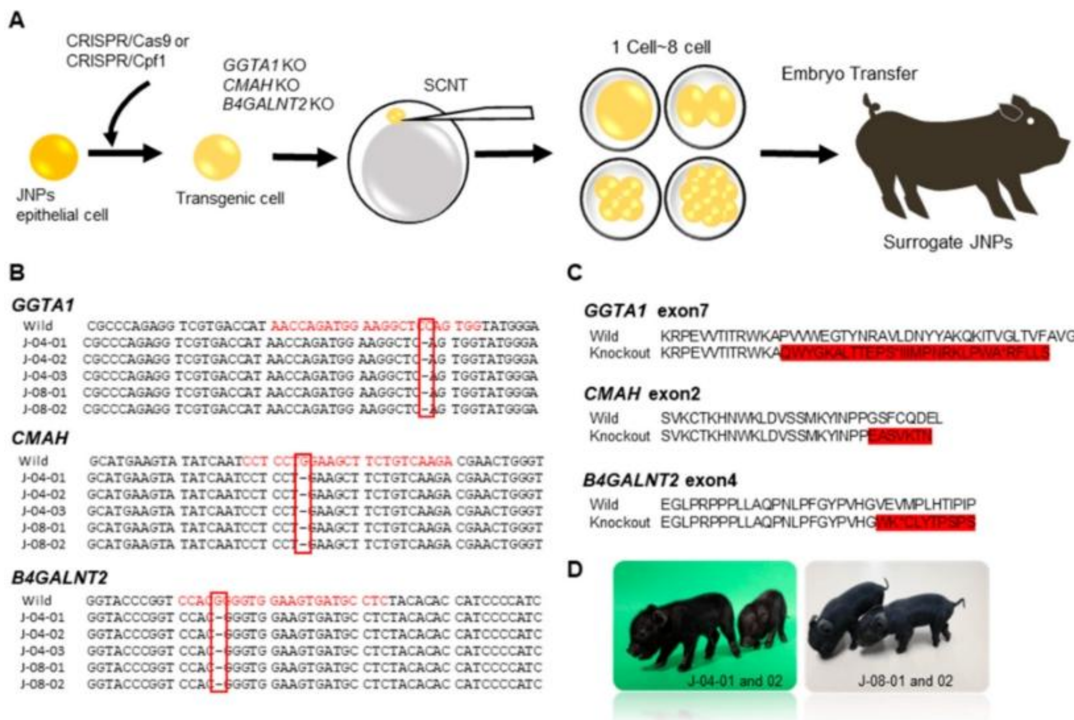


Figure 3 Schematic workflow of the generation of triple-knockout Jeju Native Pigs (JNPs), including gene editing strategies and steps required to obtain the modified JNPs (Adopted from Yoon et al., 2022)

Image caption: (A) Schematic diagram for triple-knockout development; (B) the sequences of GGTA1, CMAH, and B4GALNT2; (C) deep sequence analysis of the target region of genes in delivered piglets and details of the target sequences in exon 7 (GGTA1), exon 2 (CMAH), and exon 4 (B4GALNT2); (D) knockout piglets produced. SCNT, somatic cell nuclear transfer (Adopted from Yoon et al., 2022).

4 Enhancing Organ Longevity with CRISPR-Cas9

4.1 Genetic modifications aimed at improving organ longevity

CRISPR-Cas9 technology has facilitated significant advancements in genetic modifications to enhance organ longevity post-transplantation. Key modifications focus on increasing resistance to ischemia-reperfusion injury (IRI), reducing inflammation, and promoting tissue repair mechanisms. For instance, upregulating the expression of anti-apoptotic genes such as Bcl-2 and heme oxygenase-1 (HO-1) has proven effective in protecting cells from apoptosis and oxidative stress (Wang et al., 2018). Additionally, targeting genes involved in inflammatory pathways, such as NF-κB and its associated cytokines, can mitigate the inflammatory response and subsequent tissue damage (Kurzhagen et al., 2023).

Another critical strategy involves modulating antioxidant pathways. Enhancing the expression of genes like Nrf2, which regulates the cellular antioxidant response, has shown potential in reducing oxidative damage and improving organ function (Kurzhagen et al., 2023).

4.2 Mechanisms by which gene editing enhances organ durability

Gene editing enhances organ durability through several key mechanisms. One approach involves the upregulation of anti-apoptotic genes, such as Bcl-2, which prevents programmed cell death in transplanted tissues. This strategy is crucial for reducing cell loss during the transplantation and reperfusion phases (Bilbao et al., 1999). Another important mechanism is increasing resistance to ischemia-reperfusion injury. Upregulating genes like HO-1 and using CRISPR to knock out pro-inflammatory genes (e.g., TNF-α) helps to mitigate the effects of IRI by reducing oxidative stress, inflammation, and cellular apoptosis.

Modulating genes involved in cellular stress responses, such as Nrf2, also enhances the organ's ability to withstand oxidative stress and inflammation. For instance, CRISPR/Cas9-mediated Keap1 knockout augments

Nrf2 activity, boosting the organ's resilience (Kurzhagen et al., 2023). Additionally, gene editing can target pathways that improve overall organ function and repair mechanisms. Enhancing the expression of growth factors like GDF15 aids in tissue repair and reduces inflammation, thereby promoting long-term organ health (Liu et al., 2020).

4.3 Case studies and experimental results on organ longevity

In recent studies, CRISPR/Cas9-mediated genetic modifications have demonstrated significant potential in enhancing organ longevity. For example, research on CRISPR/Cas9-mediated Keap1 knockout in CD4+ T cells revealed increased Nrf2 antioxidant potential, leading to protection against kidney ischemia-reperfusion injury. This modification resulted in improved kidney function, reduced inflammation, and decreased cell death in T cell-deficient mice (Kurzhagen et al., 2023).

Another study involving the delivery of anti-IL-6 nanoparticles to liver transplants showed significant protection against ischemia-reperfusion injury. This approach reduced chronic rejection and inflammation, resulting in improved long-term graft survival and function (Solhjou et al., 2017). In a rat liver transplant model, the use of siRNA to inhibit apoptosis-associated genes demonstrated a significant reduction in ischemia-reperfusion injury. This treatment improved liver function and decreased pro-inflammatory cytokine levels (Bonaccorsi-Riani et al., 2022).

Research on the use of hydrogen sulfide (H2S) donor molecules to mitigate cold ischemia-reperfusion injury in solid organ transplantation also showed promising results. H2S supplementation in preservation solutions or administration to organ donors and recipients significantly reduced oxidative stress, inflammation, and cell death, thereby improving graft survival and function (Dugbartey et al., 2021).

5 Ethical and Regulatory Considerations

5.1 Ethical Issues surrounding gene editing in animals

Gene editing in animals using CRISPR-Cas9 technology raises several ethical issues. The ability to make precise genetic modifications in animals introduces concerns about animal welfare, potential ecological impacts, and the broader implications of such modifications. Ethical considerations include the risk of unintended mutations, the possibility of creating animals with enhanced traits that may suffer from unforeseen health issues, and the moral status of genetically modified animals (Rodriguez, 2016).

Moreover, the use of CRISPR-Cas9 in animals for xenotransplantation introduces additional ethical dilemmas. The primary concerns revolve around the welfare of the donor animals, the potential for cross-species disease transmission, and the ethical implications of using animals as a source of human organs (Ayanoğlu et al., 2020). Public engagement and ethical reflection are recommended to guide decision-making and ensure that gene editing applications align with societal values.

5.2 Regulatory frameworks for CRISPR-Cas9 applications in xenotransplantation

The regulatory frameworks for CRISPR-Cas9 applications in xenotransplantation vary globally but generally focus on ensuring safety and efficacy while addressing ethical concerns. In many countries, regulatory bodies treat xenotransplantation products as pharmaceutical products, requiring rigorous safety assessments and ethical reviews before clinical trials can commence. The frameworks include specific conditions about the safety of source animals, the xenotransplantation product, and the manufacturing process (Jorqui-Azofra, 2020).

For instance, in the United States, the FDA oversees the clinical applications of CRISPR-Cas9 technology, focusing on gene therapies and assisted reproductive technologies. The regulatory landscape emphasizes the importance of preclinical studies to demonstrate safety and efficacy, and the implementation of risk management protocols to mitigate potential infectious outbreaks (Grant, 2016).

Internationally, there is a call for harmonized regulatory frameworks to address the global implications of xenotransplantation. Coordinated international actions are necessary to balance individual and collective rights and to ensure public trust in the regulatory process (Jorqui-Azofra, 2020).

5.3 Public perception and acceptance of gene-edited organs

Public perception and acceptance of gene-edited organs are crucial for the successful implementation of xenotransplantation technologies. The public's attitude towards gene editing in animals and its application in medical treatments can significantly influence regulatory policies and the adoption of these technologies.

Studies indicate that public concerns primarily revolve around the ethical implications of genetic modifications and the potential risks associated with consuming genetically modified products or receiving gene-edited transplants. Transparency in the regulatory process and effective communication about the safety, benefits, and ethical considerations of gene editing are essential to gain public trust and acceptance (Rodriguez, 2016).

Engaging the public in discussions about the ethical, legal, and social implications of gene editing can foster informed decision-making and promote a shared responsibility for the technology's development. Public engagement initiatives, such as consultations and forums, can help address societal concerns and build consensus on the acceptable uses of gene editing.

6 Challenges and Limitations

6.1 Technical challenges in CRISPR-Cas9 gene editing

CRISPR-Cas9 technology, while revolutionary, faces several technical challenges that impact its efficacy and reliability. One of the primary challenges is the delivery of CRISPR components into target cells. Efficient delivery methods, such as viral vectors, liposomes, and electroporation, need to be optimized for different cell types and tissues to ensure successful gene editing (Kimberland et al., 2018). Additionally, the efficiency of homologous recombination for precise gene editing remains low, particularly in non-dividing cells, which limits the application of CRISPR-Cas9 for precise genetic modifications (Cao et al., 2016).

Another significant challenge is the specificity of CRISPR-Cas9. Designing guide RNAs (gRNAs) that precisely target the desired DNA sequence without affecting similar sequences elsewhere in the genome is complex and requires thorough validation (Doench et al., 2015). The presence of single nucleotide polymorphisms (SNPs) can also affect the binding efficiency of gRNAs, necessitating personalized approaches for gene editing in therapeutic applications (Lessard et al., 2017).

6.2 Potential off-target effects and genetic stability

One of the most critical concerns with CRISPR-Cas9 is its potential to cause off-target effects, which are unintended modifications in the genome that occur when the gRNA binds to similar but non-target sequences. These off-target mutations can lead to genomic instability and unintended phenotypic consequences, which are particularly concerning for clinical applications (Guo et al., 2023).

Several strategies have been developed to minimize off-target effects, including the use of high-fidelity Cas9 variants (e.g., SpCas9-HF1), paired nickases, and truncated gRNAs, which increase the specificity of gene editing . Additionally, advanced methods such as Digenome-seq and VIVO (Verification of In Vivo Off-targets) are employed to detect and analyze off-target effects comprehensively (Kimberland et al., 2018).

Despite these advancements, achieving complete genetic stability remains challenging. Continuous monitoring and validation of gene-edited organisms are required to ensure that off-target effects are minimized and do not compromise the intended therapeutic outcomes (Akçakaya et al., 2018).

6.3 Immunological complexities and long-term effects

The immunological response to CRISPR-Cas9 components, particularly the Cas9 protein derived from bacteria, poses significant challenges. The human immune system can recognize and mount an immune response against Cas9, leading to reduced efficacy of gene editing and potential adverse effects (Zhang et al., 2015). This immunogenicity can limit the repeated use of CRISPR-Cas9 in therapeutic applications.

Long-term effects of CRISPR-Cas9 gene editing also need to be thoroughly understood. While short-term studies have shown promising results, the long-term stability and safety of the edited genome require extensive evaluation.

Potential risks include insertional mutagenesis, where the integration of exogenous DNA disrupts important genes, and the activation of oncogenes or the inactivation of tumor suppressor genes, leading to cancer development (Jo et al., 2019).

Moreover, the durability of the therapeutic effect and the maintenance of the edited gene's function over time are critical for the success of gene editing therapies. Continuous monitoring and follow-up studies are necessary to ensure the long-term benefits and safety of CRISPR-Cas9-mediated therapies (Wienert et al., 2019).

7 Future Directions and Perspectives

7.1 Emerging trends and innovations in gene editing for organ transplantation

The field of gene editing for organ transplantation is rapidly evolving with several emerging trends and innovations. One of the significant trends is the development of CRISPR-Cas9-based multiplex gene editing, which allows simultaneous modification of multiple genes. This approach is being used to create pigs with multiple genetic modifications to reduce immunogenicity and enhance compatibility with human recipients (Eisenson et al., 2022).

Advancements in organoid technology combined with CRISPR-Cas9 are also promising. These advancements allow the creation of genetically modified organoids from pig stem cells, which can be used for detailed studies on organ development and disease modeling (Ramakrishna et al., 2021). The integration of CRISPR-Cas9 with next-generation sequencing and artificial intelligence is further enhancing the precision and efficiency of gene editing by enabling better target selection and prediction of off-target effects.

7.2 Potential breakthroughs and long-term vision for crispr-cas9 in xenotransplantation

The long-term vision for CRISPR-Cas9 in xenotransplantation includes the development of organs that are fully compatible with the human immune system and have enhanced longevity. A potential breakthrough in this field is the use of CRISPR-Cas9 to edit pig genes responsible for the expression of major xenoantigens, such as GGTA1, CMAH, and β4GalNT2, which has already shown significant progress in reducing immune rejection in preclinical models (Liu et al., 2020).

Further, the integration of CRISPR-Cas9 with gene drive technology could enable the propagation of desirable genetic traits in donor animal populations, thereby standardizing and improving the quality of xenotransplantation organs (Johnson et al., 2016). Additionally, breakthroughs in non-viral delivery systems for CRISPR-Cas9 components are likely to overcome current limitations related to immunogenicity and off-target effects, enhancing the clinical applicability of this technology (Wei et al., 2020).

7.3 Collaboration and interdisciplinary research opportunities

The future of CRISPR-Cas9 in xenotransplantation is highly dependent on interdisciplinary collaboration and research. Combining expertise from genetics, immunology, bioengineering, and clinical medicine is essential to address the complex challenges of gene editing and organ transplantation (Fung and Kerridge, 2016). Collaborative efforts can foster the development of innovative solutions for improving the safety, efficiency, and ethical considerations of CRISPR-Cas9 applications.

International consortia and partnerships between academic institutions, industry, and regulatory bodies are also crucial for advancing research and translating laboratory findings into clinical practice. These collaborations can facilitate the establishment of standardized protocols, regulatory frameworks, and public engagement strategies, ensuring the responsible use of gene editing technologies in xenotransplantation (Eisenson et al., 2022).

8 Conclusion

The exploration of CRISPR-Cas9 technology for gene editing in pigs aimed at organ transplantation has revealed significant advancements and insights. CRISPR-Cas9 enables precise genetic modifications to reduce immunogenicity, specifically by targeting genes such as GGTA1, CMAH, and β4GalNT2, which are responsible for major xenoantigens. This genetic intervention has shown promise in mitigating immune rejection and enhancing the compatibility of pig organs for human transplantation.

Moreover, strategies to enhance organ longevity through genetic modifications have been developed, focusing on increasing resistance to ischemia-reperfusion injury, reducing inflammation, and promoting tissue repair. The utilization of CRISPR-Cas9 to upregulate anti-apoptotic and antioxidant pathways, such as Bcl-2 and Nrf2, has shown potential in improving the durability and function of transplanted organs.

The findings underscore the transformative potential of CRISPR-Cas9 in xenotransplantation, paving the way for more refined and efficient genetic modifications to improve organ compatibility and longevity. Future research should focus on overcoming technical challenges, such as optimizing delivery methods and minimizing off-target effects, to enhance the precision and safety of gene editing.

Additionally, long-term studies are essential to evaluate the stability and durability of the genetic modifications and their effects on organ function over time. The development of non-viral delivery systems and high-fidelity Cas9 variants will be crucial in addressing current limitations and advancing clinical applications.

To fully realize the potential of CRISPR-Cas9 in xenotransplantation, continued interdisciplinary research and collaboration are vital. Researchers, clinicians, bioethicists, and regulatory bodies must work together to address the technical, ethical, and social challenges associated with gene editing. Ethical considerations, particularly concerning animal welfare and the potential for unintended consequences, must be integrated into the research framework to ensure responsible and sustainable advancements.

Public engagement and transparency in the research process are also crucial to build trust and acceptance of gene-edited organs. Open dialogue about the benefits, risks, and ethical implications of CRISPR-Cas9 technology will help align scientific advancements with societal values and expectations.

Acknowledgments

We would like to express our gratitude to the two anonymous peer reviewers for their critical assessment and constructive suggestions on our manuscript.

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.

References

Akçakaya P., Bobbin M.L., Guo J.A., Malagón-López J., Clement K., Garcia S., and Joung J., 2018, In vivo CRISPR editing with no detectable genome-wide off-target mutations, Nature, 561(7723): 416-419.
https://doi.org/10.1038/s41586-018-0500-9

Ali A., Kemter E., and Wolf E., 2023, Advances in Organ and Tissue Xenotransplantation, Annual Review of Animal Biosciences, 12: 369-390.
https://doi.org/10.1146/annurev-animal-021122-102606

Ayanoğlu F., Elçin A.E., and Elçin Y.M., 2020, Bioethical issues in genome editing by CRISPR-Cas9 technology, Turkish Journal of Biology, 44: 110-120.
https://doi.org/10.3906/biy-1912-52

Bilbao G., Contreras J., Eckhoff D., Mikheeva G.V., Krasnykh V., Douglas J.T., and Curiel D., 1999, Reduction of ischemia-reperfusion injury of the liver by in vivo adenovirus-mediated gene transfer of the antiapoptotic Bcl-2 gene, Annals of Surgery, 230(2): 185-193.
https://doi.org/10.1097/00000658-199908000-00008

Bonaccorsi-Riani E., Gillooly A.R., Iesari S., Brüggenwirth I., Ferguson C.M., Komuta M., and Martins P.N., 2022, Delivering siRNA Compounds During HOPE to Modulate Organ Function: A Proof-of-concept Study in a Rat Liver Transplant Model, Transplantation, 106(10): 1565-1576.
https://doi.org/10.1097/TP.0000000000004175

Cao J., Wu L., Zhang S.M., Lu M., Cheung W.C., Cai W.L., and Yan Q., 2016, An easy and efficient inducible CRISPR/Cas9 platform with improved specificity for multiple gene targeting, Nucleic Acids Research, 44, e149.
https://doi.org/10.1093/nar/gkw660

Collins P.J., Hale C., and Xu H., 2017, Edited course of biomedical research: leaping forward with CRISPR, Pharmacological Research, 125: 258-265.
https://doi.org/10.1016/j.phrs.2017.09.008

Cravedi P., 2019, Editorial: New perspectives to increase transplant numbers and survival, Current Opinion in Organ Transplantation, 24(1): 1-3.
https://doi.org/10.1097/MOT.0000000000000602

Doench J.G., Fusi N., Sullender M.E., Hegde M., Vaimberg E., Donovan K.F., and Root D., 2015, Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9, Nature Biotechnology, 34(2): 184-191.
https://doi.org/10.1038/nbt.3437

Dugbartey G.J., Juriasingani S., Zhang M.Y., and Şener A., 2021, H2S donor molecules against cold ischemia-reperfusion injury in preclinical models of solid organ transplantation, Pharmacological Research, 172: 105842.
https://doi.org/10.1016/j.phrs.2021.105842

Eisenson D.L., Hisadome Y., and Yamada K., 2022, Progress in Xenotransplantation: Immunologic Barriers Advances in Gene Editing and Successful Tolerance Induction Strategies in Pig-To-Primate Transplantation, Frontiers in Immunology, 13: 899657.
https://doi.org/10.3389/fimmu.2022.899657

Ekser B., Cooper D.K.C., and Tector A.J., 2015, The need for xenotransplantation as a source of organs and cells for clinical transplantation, International Journal of Surgery, 23(Pt B): 199-204.
https://doi.org/10.1016/j.ijsu.2015.06.066

Ekser B., Rigotti P., Gridelli B., and Cooper D., 2009, Xenotransplantation of solid organs in the pig-to-primate model, Transplant Immunology, 21(2): 87-92.
https://doi.org/10.1016/j.trim.2008.10.005

Fu R., Fang M., Xu K., Ren J., Zou J., Su L., Chen X., An P., Yu D., Ka M., Hai T., Li Z., Li W., Yang Y., Zhou Q., and Hu Z., 2020, Generation of GGTA1-/-β2M-/-CIITA-/- Pigs Using CRISPR/Cas9 Technology to Alleviate Xenogeneic Immune Reactions, Transplantation, 104(8): 1566-1573.
https://doi.org/10.1097/TP.0000000000003205

Fung R., and Kerridge I., 2016, Gene editing advance re-ignites debate on the merits and risks of animal to human transplantation, Internal Medicine Journal, 46(5): 556-559.
https://doi.org/10.1111/imj.13183

Gao H., Zhao C., Xiang X., Li Y., Zhao Y., Li Z., Pan D., Dai Y., Hara H., Cooper D., Cai Z., and Mou L., 2016, Production of α1,3-galactosyltransferase and cytidine monophosphate-N-acetylneuraminic acid hydroxylase gene double-deficient pigs by CRISPR/Cas9 and handmade cloning, The Journal of Reproduction and Development, 63: 17-26.
https://doi.org/10.1262/jrd.2016-079

Gock H., Nottle M., Lew A., D'apice A., and Cowan P., 2011, Genetic modification of pigs for solid organ xenotransplantation, Transplantation Reviews, 25(1): 9-20.
https://doi.org/10.1016/j.trre.2010.10.001

Grant E.V., 2016, FDA Regulation of Clinical Applications of CRISPR-CAS Gene-Editing Technology, Food and drug law journal 71(4): 608-633.

Guo C., Ma X., Gao F., and Guo Y., 2023, Off-target effects in CRISPR/Cas9 gene editing, Frontiers in Bioengineering and Biotechnology, 11: 1143157.
https://doi.org/10.3389/fbioe.2023.1143157

He J., Biswas R., Bugde P., Li J., Liu D.X., and Li Y., 2022, Application of CRISPR/Cas9 System to Study Biological Barriers to Drug Delivery, Pharmaceutics, 14: 50894.
https://doi.org/10.3390/pharmaceutics14050894

Hryhorowicz M., Zeyland J., Słomski R., and Lipinski D., 2017, Genetically Modified Pigs as Organ Donors for Xenotransplantation, Molecular Biotechnology, 59(5-6): 435-444.
https://doi.org/10.1007/s12033-017-0024-9

Jo D., Koo T., Cho C.S., Kim J.H., Kim J.S., and Kim J.H., 2019, Long-term effects of in vivo genome editing in the mouse retina using Campylobacter jejuni Cas9 expressed via adeno-associated virus, Molecular Therapy, 27(1): 130-136.
https://doi.org/10.1016/j.ymthe.2018.10.009

Johnson J.A., Altwegg R., Evans D.M., Ewen J., Gordon I.J., Pettorelli N., and Young J.K., 2016, Is there a future for genome-editing technologies in conservation? Animal Conservation, 19(2): 97-101.
https://doi.org/10.1111/acv.12273

Jorqui-Azofra M., 2020, Regulation of Clinical Xenotransplantation: A Reappraisal of the Legal, Ethical, and Social Aspects Involved, Methods in molecular biology, 2110: 315-358.
https://doi.org/10.1007/978-1-0716-0255-3_20

Kimberland M., Hou W., Alfonso-Pecchio A., Wilson S., Rao Y., Zhang S., and Lu Q., 2018, Strategies for controlling CRISPR/Cas9 off-target effects and biological variations in mammalian genome editing experiments, Journal of Biotechnology, 284: 91-101.
https://doi.org/10.1016/j.jbiotec.2018.08.007

Klymiuk N., Aigner B., Brem G., and Wolf E., 2010, Genetic modification of pigs as organ donors for xenotransplantation, Molecular Reproduction and Development, 77(3): 209-221.
https://doi.org/10.1002/mrd.21127

Kurzhagen J., Noel S., Lee K., Sadasivam M., Gharaie S., Ankireddy A., and Rabb H., 2023, T cell Nrf2/Keap1 gene editing using CRISPR/Cas9 and experimental kidney ischemia-reperfusion injury, Antioxidants and Redox Signaling, 38(13-15):959-973.
https://doi.org/10.1089/ars.2022.0058

Längin M, Mayr T, Reichart B, Michel S, Buchholz S, Guethoff S, Dashkevich A, Baehr A, Egerer S, Bauer A, Mihalj M, Panelli A, Issl L, Ying J, Fresch AK, Buttgereit I, Mokelke M, Radan J, Werner F, Lutzmann I, Steen S, Sjöberg T, Paskevicius A, Qiuming L, Sfriso R, Rieben R, Dahlhoff M, Kessler B, Kemter E, Kurome M, Zakhartchenko V, Klett K, Hinkel R, Kupatt C, Falkenau A, Reu S, Ellgass R, Herzog R, Binder U, Wich G, Skerra A, Ayares D, Kind A, Schönmann U, Kaup FJ, Hagl C, Wolf E, Klymiuk N, Brenner P, Abicht J.M., Consistent success in life-supporting porcine cardiac xenotransplantation, Nature, 564(7736): 430-433.
https://doi.org/10.1038/s41586-018-0765-z

Lei T., Chen L., Wang K., Du S., Gonelle-Gispert C., Wang Y., and Buhler L., 2022, Genetic engineering of pigs for xenotransplantation to overcome immune rejection and physiological incompatibilities: The first clinical steps, Frontiers in Immunology, 13: 1031185.
https://doi.org/10.3389/fimmu.2022.1031185

Lessard S., Francioli L., Alfoldi J., Tardif J., Ellinor P., MacArthur D., and Lettre G., 2017, Human genetic variation alters CRISPR-Cas9 on- and off-targeting specificity at therapeutically implicated loci, Proceedings of the National Academy of Sciences, 114(52): 11257-11266.
https://doi.org/10.1073/pnas.1714640114

Li C., Brant E.J., Budak H., and Zhang B., 2021, CRISPR/Cas: a Nobel Prize award-winning precise genome editing technology for gene therapy and crop improvement, Journal of Zhejiang University, Science, 22: 253-284.
https://doi.org/10.1631/jzus.B2100009

Li M., Guo X., Cheng L., Zhang H., Zhou M., Zhang M., Yin Z., Guo T., Zhao L., Liu H., 2024, Porcine Kidney Organoids Derived from Naïve-like Embryonic Stem Cells, International Journal of Molecular Sciences, 25(1): 682.
https://doi.org/10.3390/ijms25010682

Liu J., Kumar S., Heinzel A., Gao M., Guo J., Alvarado G.F., and McMahon A.P., 2020, Renoprotective and Immunomodulatory Effects of GDF15 following AKI Invoked by Ischemia-Reperfusion Injury, Journal of the American Society of Nephrology, 31(4): 733-748.
https://doi.org/10.1681/ASN.2019090876

Liu X., Wu S., Xu J., Sui C., and Wei J., 2017, Application of CRISPR/Cas9 in plant biology, Acta Pharmaceutica Sinica, 7: 292-302.
https://doi.org/10.1016/j.apsb.2017.01.002

Liu Y., Qin L., Tong R., Liu T., Ling C., Lei T., Zhang D., Wang Y., and Deng S., 2020, Regulatory changes in China on xenotransplantation and related products, Xenotransplantation, 27(3):e12601.
https://doi.org/10.1111/xen.12601

Lowder L.G., Zhang D., Baltes N., Paul J.W., Tang X., Zheng X., and Qi Y., 2015, A CRISPR/Cas9 toolbox for multiplexed plant genome editing and transcriptional regulation, Plant Physiology, 169: 971-985.
https://doi.org/10.1104/pp.15.00636

Mollanoori H., and Teimourian S., 2018, Therapeutic applications of CRISPR/Cas9 system in gene therapy, Biotechnology Letters, 40: 907-914.
https://doi.org/10.1007/s10529-018-2555-y

Niu D., Wei H., Lin L., George H., Wang T., Lee I.H., and Yang L., 2017, Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9, Science, 357(6357): 1303-1307.
https://doi.org/10.1126/science.aan4187

Ramakrishna G., Babu P.E., Singh R., and Trehanpati N., 2021, Application of CRISPR-Cas9 based gene editing to study the pathogenesis of colon and liver cancer using organoids, Hepatology International.
https://doi.org/10.1007/s12072-021-10237-z

Ratan Z.A., Son Y.J., Haidere M.F., Uddin B.M.M., Yusuf M.A., Zaman S.B., and Cho J., 2018, CRISPR-Cas9: a promising genetic engineering approach in cancer research, Therapeutic Advances in Medical Oncology, 10: 1758834018755089.
https://doi.org/10.1177/1758834018755089

Rodriguez E., 2016, Ethical Issues in Genome Editing using Crispr/Cas9 System, Journal of Clinical Research and Bioethics, 7: 1-4.

Rodríguez E., 2017, Ethical Issues in Genome Editing for Non-Human Organisms Using CRISPR/Cas9 System, Journal of Clinical Research and Bioethics, 8: 1-5.
https://doi.org/10.4172/2155-9627.1000300

Solhjou Z., Uehara M., Bahmani B., Maarouf O.H., Ichimura T., Brooks C.R., and Abdi R., 2017, Novel Application of Localized Nanodelivery of Anti-Interleukin-6 Protects Organ Transplant From Ischemia-Reperfusion Injuries, American Journal of Transplantation, 17(9): 2326-2337.
https://doi.org/10.1111/ajt.14266

Sun D., Guo Z., Liu Y., and Zhang Y., 2017, Progress and Prospects of CRISPR/Cas Systems in Insects and Other Arthropods, Frontiers in Physiology, 8: 608
https://doi.org/10.3389/fphys.2017.00608

Tanihara F., Hirata M., Nguyen N.T., Sawamoto O., Kikuchi T., and Otoi T., 2021, One-Step Generation of Multiple Gene-Edited Pigs by Electroporation of the CRISPR/Cas9 System into Zygotes to Reduce Xenoantigen Biosynthesis, International Journal of Molecular Sciences, 22(5): 2249.
https://doi.org/10.3390/ijms22052249

Wang R., Ruan M., Zhang R., Chen L., Li X., Fang B., and Dai Y., 2018, Antigenicity of tissues and organs from GGTA1/CMAH/β4GalNT2 triple gene knockout pigs, Journal of Biomedical Research, 33(4): 235-243.
https://doi.org/10.7555/JBR.32.20180018

Wang R., Ruan M., Zhang R., Chen L., Li X., Fang B., Li C., Ren X., Liu J., Xiong Q., Zhang L., Jin Y., Li L., Li R., Wang Y., Yang H., and Dai Y., 2018, Antigenicity of tissues and organs from GGTA1/CMAH/β4GalNT2 triple gene knockout pigs, Journal of Biomedical Research, 33(4): 235-243.
https://doi.org/10.7555/JBR.32.20180018

Wei T., Cheng Q., Farbiak L., Anderson D.G., Langer R., and Siegwart D., 2020, Delivery of Tissue-Targeted Scalpels: Opportunities and Challenges for In Vivo CRISPR/Cas-Based Genome Editing, ACS Nano, 14(8): 9243-9262.
https://doi.org/10.1021/acsnano.0c04707

Wienert B., Wyman S.K., Richardson C.D., Yeh C.D., Akcakaya P., Porritt M.J., and Corn J.E., 2019, Unbiased detection of CRISPR off-targets in vivo using DISCOVER-Seq, Science, 364(6437): 286-289.
https://doi.org/10.1126/science.aav9023

Wolf E., Kemter E., Klymiuk N., and Reichart B., 2019, Genetically modified pigs as donors of cells tissues, and organs for xenotransplantation, Animal Frontiers: The Review Magazine of Animal Agriculture, 9(2): 13-20.
https://doi.org/10.1093/af/vfz014

Wu H., Lian M., and Lai L., 2023, Multiple gene modifications of pigs for overcoming obstacles of xenotransplantation, National Science Open, 5(2): 17.
https://doi.org/10.1360/nso/20230030

Yoon S., Lee S., Park C., Choi H., Yoo M., Lee S.C., Hyun C.H., Kim N., Kang T., Son E., Ghosh M., Son Y.O., and Hur C., 2022, An Efficacious Transgenic Strategy for Triple Knockout of Xeno-Reactive Antigen Genes GGTA1, CMAH, and B4GALNT2 from Jeju Native Pigs, Vaccines, 10(9): 1503.
https://doi.org/10.3390/vaccines10091503

Zhang R., Wang Y., Chen L., Wang R., Li C., Li X., Fang B., Ren X., Ruan M., Liu J., Xiong Q., Zhang L., Jin Y., Zhang M., Liu X., Li L., Chen Q., Pan D., Li R., Cooper D., Yang H., and Dai Y., 2018, Reducing immunoreactivity of porcine bioprosthetic heart valves by genetically-deleting three major glycan antigens, GGTA1/β4GalNT2/CMAH, Acta Biomaterialia, 72: 196-205.
https://doi.org/10.1016/j.actbio.2018.03.055

Zhang X., Wang Q., Zhao J., Li X., Peng W., Yang Z., Lin Z., Yang L., Ding R., Tao K., and Dou K., 2020, The resurgent landscape of xenotransplantation of pig organs in nonhuman primates, Science China Life Sciences, 64(6): 697-708.
https://doi.org/10.1007/s11427-019-1806-2

Zhang X.H., Tee L.Y., Wang X.G., Huang Q.S., and Yang S.H., 2015, Off-target effects in CRISPR/Cas9-mediated genome engineering, Molecular Therapy - Nucleic Acids, 4: e264.
https://doi.org/10.1038/mtna.2015.37

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