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Review Article

CRISPR/Cas-edited pigs for personalized medicine: more than preclinical test-system

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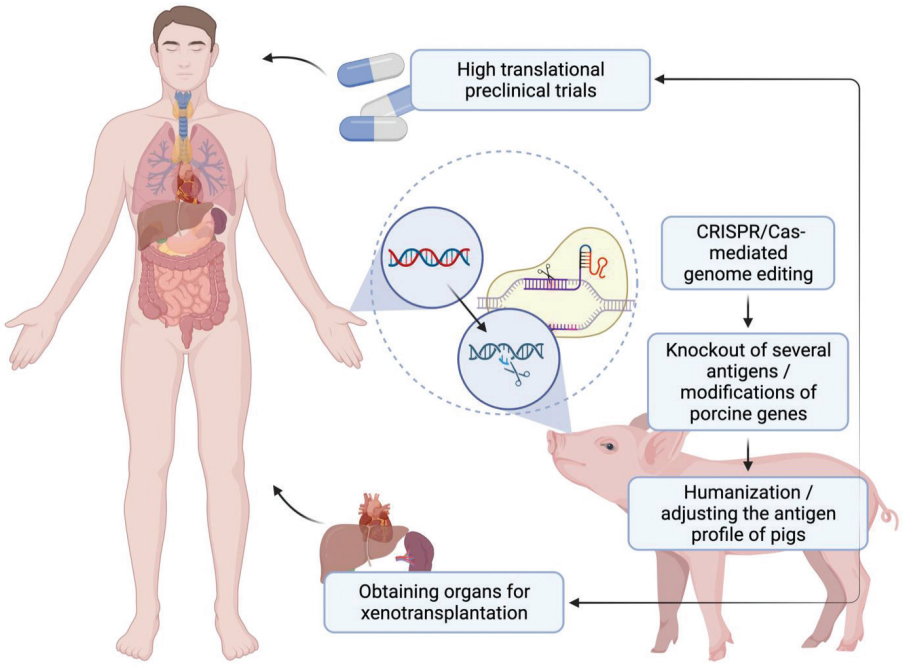
Abstract

Novel CRISPR-Cas-based genome editing tools made it feasible to introduce a variety of precise genomic modifications in the pig genome, including introducing multiple edits simultaneously, inserting long DNA sequences into specifically targeted loci, and performing nucleotide transitions and transversions. Pigs serve as a vital agricultural resource and animal model in biomedical studies, given their advantages over the other models. Pigs share high similarities to humans regarding body/organ size, anatomy, physiology, and a metabolic profile. The pig genome can be modified to carry the same genetic mutations found in humans to replicate inherited diseases to provide preclinical trials of drugs. Moreover, CRISPR-based modification of pigs antigen profile makes it possible to offer porcine organs for xenotransplantation with minimal transplant rejection responses. This review summarizes recent advances in endonuclease-mediated genome editing tools and research progress of genome-edited pigs as personalized test-systems for preclinical trials and as donors of organs with human-fit antigen profile.

Graphical abstract:



Keywords

CRISPR-Cas, personalized models, xenotransplantation, pig, blastocyst complementation, animal model

Introduction

Rapid advances in genome editing have made it possible to quickly and accurately create new animal models required to study the pathogenesis of human diseases and discover new pharmacological targets. Obviously, these revolutionary genome editing technologies have opened up new avenues for the design and development of promising novel human therapies. However, although some of the possible applications of genome editing technologies have been successfully introduced in the laboratories worldwide, some of them are still a long way off. For instance, genetically modified mice are the organisms routinely used to obtain new data on mammalian physiology, disease pathogenesis and to test new medicals (Bruter et al. 2021; Dolskiy et al. 2022). Some of species like rabbits and goats were proposed to generate bioreactors producing human proteins for pharmaceutics (Goldman et al. 2012; Maksimenko et al. 2013; Gurskiy et al. 2016). Nevertheless, despite numerous benefits for pharmacology and medicine, there are still a very limited number of studies focused on generation of genetically modified pigs.

In this review, we summarize recent advances in endonuclease-mediated genome editing tools, the research progress of genome-edited pigs as models for nonclinical trials and as organ donors for xenotransplantation highlighting the prospects of their clinical applications.

Gene editing tools

The traditional gene targeting technique developed in mice required homologous recombination (HR) and manipulation of embryonic stem cells (ES) (Mansour et al. 1988). Due to the lack of "real" ES-cells, this technique has never been applicable in livestock species. The development of new synthetic, precise tools for the genetic modification of mammalian genomes, such as ZFN, TALENs, and CRISPR-Cas, rendered complex genetic modifications feasible and efficient in non-rodent mammals (Fig.1). The ability to introduce a single double-strand break (DSB) at a unique predetermined site enables genes to be inactivated by insertion or deletion mutations (Indels) via non-homologous end-joining (NHEJ) repair or by targeted sequence replacement via homology-directed repair with an exogenous homologous DNA or single-stranded oligonucleotide fragment. The practicality and simplicity of gene editing have steadily improved in successive generations of endonuclease systems beginning with Zinc finger nucleases (ZFN) (Hauschild et al. 2011; Kwon et al. 2013), then transcription activator-like effector nucleases (TALENs) (Carlson et al. 2012, 2014) and, most recently, the CRISPR-Cas9 system. The CRISPR-Cas9 system has substantially revolutionized and accelerated the process of knocking out endogenous genes or knocking-in exogenous sequences at targeted sites within the genome (Gaj et al. 2013). With these improved skills and efficiencies of genetic modifications, the modifications of the pig genome can confer any desired, predetermined genetic changes. Protein-guided ZFN and TALEN, as well as the RNA-guided CRISPR-Cas9 (clustered regularly interspaced short palindromic repeat-associated protein 9) system have been successfully used for the establishment of genetically engineered pig lines (Tan et al. 2016). ZFNs and TALENs have now largely been superseded by CRISPR-Cas9, which is equally, if not more, efficient in inducing DSBs and in stimulating HR (Mali et al. 2013). The CRISPR-Cas9 system and, especially, its diverse derivates also offer improved target specificity, less off-target activity, and better prediction of off-target effects (Mali et al. 2013; Fu et al. 2014; Coelho et al. 2020). Highly efficient gene editing makes it possible to carry out genetic modification directly in zygotes and early-stage embryos and thus avoiding nuclear transfer, which needs a lot expertise and equipment. Since the discovery of DNA endonucleases, there have been numerous reports of potential porcine models of human diseases based on gene knockouts generated by the injection of CRISPR-Cas9 components into zygotes (Hai et al. 2014; Tanihara et al. 2019a; Koppes et al. 2020).

Production of gene-edited livestock

Gene transfer into the porcine genome was first carried out in 1985 via DNA microinjection into the pronuclei of fertilized oocytes (Hammer et al. 1985). Since then, sperm-mediated gene transfer, lentiviral transgenesis, transposon-based transgenesis, somatic cell nuclear transfer from genetically modified cells, as well as gene editing techniques based on designer nucleases, were used for the generation of mutant pig lines (Lai et al. 2002; Lavitrano et al. 2002; Ramsoondar et al. 2003; Dieckhoff et al. 2007; Ivics et al. 2014). Porcine embryonic stem (ES) cells or induced pluripotent stem cells showing comparable developmental capacity as mouse ES cells are not yet available (Soto and Ross 2016). This might change with the recent development of porcine expanded pluripotent stem cells (EPSCs), which have the potential to differentiate into all three germ layers (Gao et al. 2019). Their complete potential for genome editing in pigs still must be explored, but might be an essential part in the

future. Genetic modification of animals is mainly achieved by germline modification of the genome. Animals with germline modifications are used to produce stable mutant lines, making the model permanently and reproducibly available during all developmental stages. Genetically engineered animals are either transgenic animals harboring experimentally transferred DNA sequences or genetically modified animals without integrating foreign DNA, but a modification of endogenous gene sequences.

Genetic engineering of animals involves projects to analyze additional functions by over-and/or ectopic expression of a transgene and/or partial or complete loss of function of endogenous genes. This includes the inactivation of specific genomic sequences (knockout), defined genomic modifications (knock-in), specific suppression of the synthesis of gene products (knockdown, gene silencing), as well as random mutagenesis of the host genome (insertional mutagenesis).

Zygote modification (microinjection, electroporation)

Porcine in vivo produced embryos and embryos following in vitro fertilization (IVF) of in vitro matured (IVM) oocytes derived from slaughterhouse ovaries are used for zygote microinjection. Basal microinjection of DNA sequences as transgenes into the pronuclei of fertilized oocytes is still carried out in mice, but generally results in low efficiencies of transgene integration, random integration of multiple transgene copies, including the rearrangement of the host genome around the integration site, insertional mutagenesis, high numbers of transgenic mosaic founders, and possible genome position effects on the expression of the transgene (Ding et al. 2005; Kumar et al. 2009; Ivics et al. 2014; Li et al. 2014). The microinjection into the pronucleus of porcine zygotes is a much more difficult endeavor, as pig oocytes contain a high content of lipids, giving the cytoplasm a dark color, thus making it impossible to visualize the pronuclei without centrifugation of the embryos (Gil et al. 2017). Intracytoplasmic microinjection became a feasible option to genetically modify the genome of early pig embryos with the development of new vectors, such as transposon-based vectors and the CRISPR-Cas system (Fig 1.). The zygote first division is a critical time point to avoid mosaicism in the obtained offspring. Therefore, the use of Ribonucleic-protein complexes (gRNA + Cas-protein) is usually preferred, as they are immediately active and remain active only for a short time, which further reduces the risk of causing undesired mutations of the genome (off-targets) (Vakulskas and Behlke 2019; Naeem et al. 2020). Recently, electroporation of zygotes has been used for generating knock-out piglets. The efficiency of genome editing (especially, biallelic) after electroporation was significantly higher than after microinjection (Le et al. 2020). This method was used to create myostatin knock-out pigs (Wang et al. 2015), a pig model for diabetes with *PDX-1* mutations (Tanihara et al. 2019b), *CD163*-edited pig (Tanihara et al. 2021b), pigs with point mutation in *KRAS* gene (Wittayarat et al. 2021), and pigs with targeted mutations in *GGTA1*, *CMAH*, and *B4GALNT2* genes, generated by one-step electroporation (Tanihara et al. 2021a). Electroporation of porcine zygotes reaches biallelic editing efficiencies of up to 70%, depending on the Cas9 concentration and the target gene (Tanihara et al. 2019a, 2021a).

Somatic cell nuclear transfer

Somatic cell nuclear transfer (SCNT, cloning) is carried out to produce pigs carrying mutations (knock-in, knock-out) in defined loci of the porcine genome after using genome editing tools (Betthauser et al. 2000; Onishi et al. 2000). The production of genetically modified animals by SCNT includes the transfection and selection of somatic donor cells in vitro, recovery and enucleation of recipient metaphase II oocytes, transfer of genetically modified somatic donor cells into the perivitelline space of the enucleated oocyte, electrical fusion of the donor cell and the oocyte, electrical activation of the reconstructed oocytes, and surgical embryo transfer to hormonally synchronized recipients (Hölker et al. 2005). In the case when genetically modified donor cells are employed, 100% genetically modified founder animals without genetic mosaicism are obtained. Usually, 80- 400 embryos are transferred per recipient (Fig.1), but higher numbers of transferred embryos do not result in larger litters. The pig cloning efficiency is varying within relatively low values between 0.5–5% (offspring/transferred cloned embryos) (Betthauser et al. 2000; Onishi et al. 2000; Kolber-Simonds et al. 2004; Hölker et al. 2005). The successful embryonic, fetal, and neonatal development of the transferred embryos derived from SCNT depends on the correct epigenetic reprogramming of the donor cell nucleus. Insufficient epigenetic reprogramming may lead to an overall low cloning efficiency, as well as to peri- and neonatal health problems of cloned pigs, which might result in 50% stillborn piglets or piglets that die perinatally (own not published results). Abnormal phenotypes of cloned pigs occur less frequently than in other cloned mammals, and they are not transmitted to the offspring of affected clones (Estrada et al. 2007). Various somatic cell types were successfully used in the cloning procedure and numerous technical variations were established to increase the efficiency of porcine SCNT (Hölker et al. 2005; Kurome et al. 2013).

Pigs as a test-system for preclinical trials

In general, because of their human-like anatomy, pigs are considered a very good test-system for pharmacological studies (Glauser 1966). High similarity of body size, mass indexes of internal organs and other physiological parameters between pigs and human beings, along with

their similar diet, determine the similarity of absorption, distribution, metabolism, and excretion (ADME) (Kararli et al. 1995; Heinritz et al. 2013). Moreover, big body size permits to test the same routes for drug administration as in real patients. Anticipatedly, porcine models are highly more powerful than the rodent ones to translate the obtained data in clinical practice. However, on the other hand, porcine models are not mainstay because they are expensive and time-consuming.

Obviously, genetically modified pigs are not routine laboratory animals. Nowadays, the most widespread application of CRISPR/Cas-edited pigs is a field of oncological studies (Xu et al. 2019). Tumorigenesis in pigs resembles that in humans, which was first shown via autologous transplantation of primary porcine cells transformed with viral oncogenic cDNAs (Adam et al. 2007). Flisikowska et al. (2012, 2017). generated pigs that carry a translational stop signal at codon 1311 in porcine APC (APC1311), which is orthologous to the human APC1309 mutation responsible for a severe form of familial adenomatous polyposis. These pigs develop polyps in the colon and rectum. As early as 4 months of age, these pigs were shown to display a clinical picture of the adenoma-carcinoma sequence. The same research group also generated pigs that carry a Cre-dependetly activated TP53R167H mutation in exon 5. Pigs heterozygous for the uninduced allele develop osteosarcomas after 16 months of age, while homozygotes show multiple osteosarcomas at 7–8 months of age (Leuchs et al. 2012; Saalfrank et al. 2016).

Additionally, CRISPR/Cas-mediated genome editing technologies were also utilized to generate porcine models of diabetes mellitus, Alzheimer's disease, Duchenne muscular atrophy, and cystic fibrosis (reviewed in Perleberg et al. 2018).

Pigs as organ donors

The growing shortage of available organs for allotransplantation has prompted scientists to search for alternative ways to treat end-stage organ failure. Pigs are now considered the optimal organ donor for xenotransplantation. The advantages of using pigs are that they share similar anatomical and physiological characteristics with humans, including cardiovascular, urinary, integumentary, and the digestive systems (Swindle et al. 2012; Ribitsch et al. 2020). Compared to other farm animals, pigs reach early puberty (at 5–8 months of age), breed well throughout the year, and have a significant number of offspring (an average of 10–14 piglets per litter) (Aigner et al. 2010; Ribitsch et al. 2020). Due to these characteristics, and the size of animals (young pigs have a size and body weight similar to humans), genetically modified pigs are widely used as models for xenotransplantation (Kahn et al. 1988; Chari et al. 1994; Martin et al. 1999; Spetzler et al. 2016; Vogel et al. 2017), human diseases and as a drug discovery platform (Dai et al. 2002; Swindle et al. 2012).

Generation of human organs in gene-edited pigs

One strategy to overcome the shortage of donor organs is the production of interspecies chimaeras by blastocyst complementation (Fig. 1). This allows obtaining both functional xenogeneic organs and tissues. The essence of the method lies in the fact that induced pluripotent stem cells (iPSCs) from a patient or a healthy donor are introduced into the pre-implantation embryo at the blastocyst stage, which theoretically should lead to the contribution of the donor stem cells to tissues and organs (Suchy and Nakauchi 2017). The use of zygotes homozygous for a knockout of a specific gene, which is evident for the formation of tissues or whole organs in recipient embryos, creates a tissue niche in blastocysts, which allows the formation of the corresponding organ from injected iPSCs. Through natural development in the embryo, all cell populations develop independently and lead to the development of specified organs and tissues during the embryo's normal development. This technology, created by Chen et al. (1993) has been shown to support organ formation after transferring normal heterologous pluripotent cells within and even between closely related species (Nagashima and Matsunari 2016). However, human iPSCs only made minor contributions to pig organs, mainly because pigs and humans are two distinct species that separated about 80 million years ago (Groenen et al. 2012). This results in distinct differences in the mechanisms of embryonic/fetal development, size and growth of the fetus, the timing of implantation, cell division rate, placental structure and so forth. Besides, pig cells will still contribute to the target human organ and form the endothelium. Therefore, such an organ would require genetic modification of the host endothelium by altering the pig embryo's genome or forming another niche that allows human stem cells to form the host's endothelium. Otherwise, the organ will trigger severe immune rejections (Freedman 2018). Pig embryos deficient in the *etv2* gene, the main regulator of hematoendothelial cells, might help to overcome this problem. After injection of human iPSCs into *etv2*-KO blastocysts, all vascular endothelial cells originated from human cells in day 17/18 embryos (Das et al. 2020). Another ethical concern remains the contribution of human cells to the parts of the pig other than the anticipated organ. For example, *SALL1* knockout pig embryos were used to create a niche for human kidney formation. It is well known that the *SALL1* protein plays a crucial role in the prenatal development of the kidneys and the hands, ears, anus, and other parts of the body (Choi et al. 2010). Thus, "wild-type" human iPSCs could contribute not only to the established organogenic niche, but might also contribute to other tissues and organs of the pig. Another work has shown more encouraging results using the blastocyst complementation method. The injection of porcine eGFP positive blastomeres into *Hhex* knockout porcine blastocysts resulted in a high degree of chimerism in the developing liver in day 25 fetuses (Ruiz-Estevez et al. 2021). The blastocyst complementation works to a certain extent

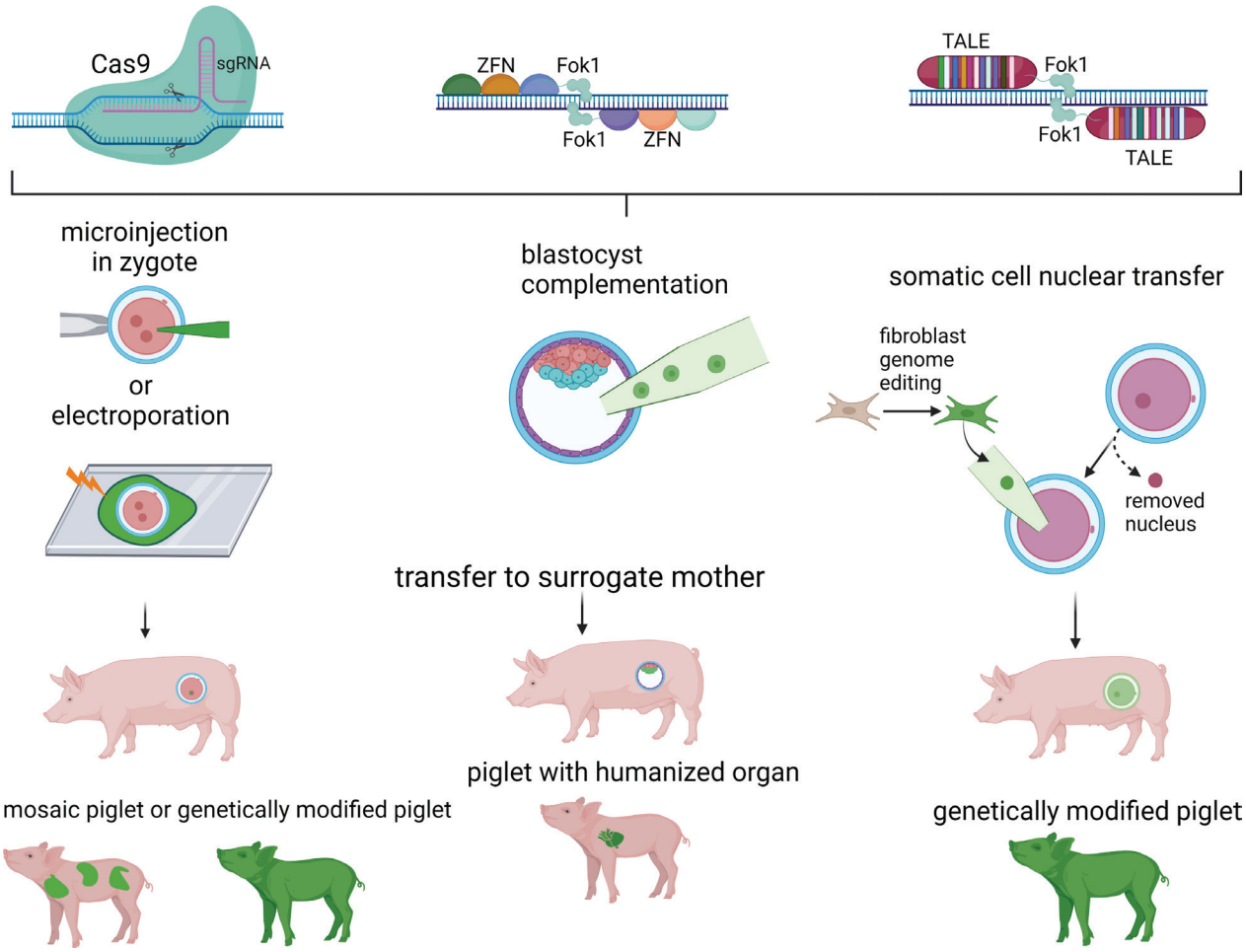


Figure 1. Approaches used to generate genetically modified pigs. The ZFN, TALEN, and CRISPR/Cas9 systems enable efficient gene targeting for modification of mammalian genomes. Microinjection or electroporation of ZFN/TALEN/CRISPR/Cas9 constructions results in one-step generation of genetically modified pigs. Interspecies blastocyst complementation is used for generation of human organs in pigs. The transfer of genetically modified somatic donor cells into oocyte is performed by electrical fusion of donor cell and oocyte. The surgical embryo transfer to hormonally synchronized recipients is necessary to receive genetically modified piglets. Created with BioRender.com.

within closely related species. Still, however, the xenogeneic barrier is a major limitation, which is still poorly understood, and hinders progress in interspecies blastocyst complementation research. Further research in this area may one day lead to the cultivation of human organs inside animals for transplantation purposes. Nevertheless, blastocyst complementation remains unrealistic to supply enough transplantable organs to be an essential solution to the shortage of donor organs. This critical view is based on two columns: 1) the generated human organ has to be genetically edited to serve as a universal donor organ not causing rejection responses that cannot be addressed by immunosuppression, 2) every single organ would have to be produced by the very inefficient and currently unachievable way of blastocyst complementation as these pigs cannot be propagated by breeding or somatic cell nuclear transfer. Therefore, this research is somewhat of more interest to study developmental biology and the function of genes than a potential solution to the growing demand for transplantable organs.

Gene-edited pigs for xenotransplantation

Xenotransplantation could undoubtedly provide substantial benefits for human regenerative medicine. However, significant immunological and physiological incompatibilities must be addressed before xenotransplants becoming clinically effective xenogeneic grafts. Fortunately, our understanding of these barriers is multiplying, and rational strategies based on genome editing were developed to surmount them. Humoral rejection by preformed antibodies and incompatibilities between the blood coagulation systems present the most immediate obstacles. The most significant challenge comes from the adaptive immune response in the long term. An unmodified porcine organ transplanted into a human or primate recipient is confronted with a series of rejection responses. The first rejection response is the hyperacute rejection (HAR), followed by the acute humoral xenograft rejection (AHXR), also known as acute vascular rejection or delayed xenograft rejection. Both HAR and

AHXR are the ultimate result of antibodies binding to graft endothelial cell surface antigens.

In HAR, preformed antibodies in the human blood against the α1,3-galactosyl-galactose (αGal) epitope on the porcine endothelium cause the rapid formation of an antigen-antibody complex, which immediately activates the host complement system. The formation of the membrane attack complex (MAC) results in extensive hemorrhage, oedema, and thrombosis of small blood vessels, leading to the destruction of the graft within minutes to hours. The finding that αGal epitopes are not present in old-world primates (including humans) due to the non-functional α1,3-galactosyltransferase (*GGTA1*, also referred to as *α1,3GT*) gene, indicated the option to disable the orthologous gene in the porcine genome. Since the first reports of *GGTA1* knockout in pigs (Dai et al. 2002; Lai et al. 2002), homozygous deficient animals have been generated (Phelps et al. 2003), and several independent herds have been established (Klymiuk et al. 2010). The benefit of organs from these pigs has been tested in numerous studies using pig-to-baboon organ transplantation models (Ekser et al. 2012) and revealed maximum survival times of three months for kidneys (Yamada et al. 2017), and 183 and 264 days after orthotopic transplantation of a pig heart (Längin et al. 2018; Mohiuddin et al. 2021).

AHXR presents the next immunological obstacle once HAR has been overcome. Hearts from *αGal*-KO pigs transplanted into baboons exhibited widespread thrombotic microangiopathy, ischemia, focal hemorrhage, and necrosis because of progressive humoral rejection and disordered thromboregulation (Ezzelarab et al. 2009). The underlying mechanisms are incompletely understood, but the binding of non-Gal antibodies and activation of the endothelium played a crucial role, leading to a procoagulant state (Diswall et al. 2010; Byrne et al. 2011). The major non-Gal antigens are Neu5GC encoded by the *CMAH* gene (Cytidine monophospho-N-acetylneuraminic acid hydroxylase), and Sda, a product of *B4GalNT2* (Beta-1,4 N-acetylgalactosaminyltransferase 2) (Padler-Karavani et al. 2008; Byrne et al. 2018). Therefore, the common sense in the xenotransplantation field is that knockouts of *GGTA1*, *CMAH* and *B4GalNT2* are essential to achieve long-term survival after xenotransplantation. This is further supported by the increasing survival times after pig-to-baboon xenotransplantations of pig hearts carrying a triple or quadruple knockout (including knockout of the porcine growth hormone receptor (GHR) to keep pigs smaller). Various transgenic pigs expressing human transgenes on the vascular endothelium have been generated and tested. Transgenes that modulate endothelial activation in the xenograft, e.g. heme oxygenase 1 (HO-1) or tumor necrosis factor-α-induced protein (A20), have been produced and have proven their efficacy to protect the endothelium from being activated after xenoperfusion with human blood (Oropeza et al. 2009; Petersen et al. 2011). In addition to the antibody-mediated activation of the xenograft endothelium, incompatibilities in the coagulation components in the human bloodstream and the porcine vessel wall have been found to contribute to the formation of microthrombi (Cowan 2007). Expression of human regulators of blood coagulation, such as tissue factor pathway inhibitor (TFPI), endothelial protein C receptor (EPCR) or thrombomodulin (THBD), can help overcome these incompatibilities (Petersen et al. 2009).

The requirements continue to be refined as knowledge increases, but the current scientific agreement on genetic modifications required in donor pigs to combat HAR and AHXR are:

- Removal of the major xenoreactive surface antigens: αGal and non-Gal epitopes Neu5GC and Sda
- One or more abundantly expressed human complement regulatory genes: e.g. *CD46*, *CD55*, *CD59*
- A human anti-thrombotic and/or anticoagulant gene: e.g. *THBD*, *EPCR*, and others
- A gene with vascular protective properties: e.g. *HO-1*, *hA20*, and others.

Porcine endogenous retroviruses (PERVs) are gamma retroviruses expressed in various organs and found in the genome of all pig lines (Bittmann et al. 2012). Compared to other known retroviruses, such as the human immunodeficiency virus (HIV) and human T-cell lymphotropic virus, integration of PERVs into the patient's genome has the potential to lead to immunodeficiency and tumorigenesis (Liu et al. 2020). No evidence of PERV transmission has been found in preclinical trials of porcine cell and organ transplantation in non-human primates and in clinical trials of transplantation of encapsulated porcine islets (Specke et al. 2001; Denner 2021). The reason for such discrepancies is not completely clear; however, the existing preclinical and clinical trials have a number of limitations (lack of functional PERV receptors in most model animals, the use of encapsulated islet cells in human clinical trials) regarding the interpretation of PERV transmission and, thus, cannot give an accurate answer about the safety of xenotransplantation (Denner 2018, 2021). Various strategies have been used to inactivate PERVs, including antiretroviral drug administration, vaccination and RNA interference (Denner 2021), but complete inactivation was only achieved with CRISPR-Cas9 in 2017 (Niu et al. 2017). Twenty-five copies of functional PERVs in a porcine primary cell line and generated PERV-inactivated pigs via somatic cell nuclear transfer have been studied. Pigs with all PERVs inactivated can also be genetically engineered to eliminate transplant rejection and these pigs exhibit normal physiology, fertility and germline transmission and cannot be infected with PERVs from other pigs including their mother (Yue et al. 2021). Taken together, new knowledge and advances in the field of porcine viruses and pathogens indicate that there are no fundamentally insoluble problems for infection safety in xenotransplantation.

Conclusions and discussion

New technologies for targeted modification of the animal genome considerably facilitate the creation of human disease models for preclinical research and gene therapy.

Remarkable results were obtained in mouse models, making it possible to obtain more advanced models of large animals, including genetically modified pigs. However, genome editing of pigs opens up even more significant avenues for medicine. The feasibility to use genetically modified pigs as donors can be a silver bullet to solve one of the dramatic problems with the deficiency of organs for transplantation.

Restoration and replacement of damaged tissues and organs are some of the priorities in the development of health care. Human tissues and organs, despite their advantages, could not overcome the global acute shortage of donor materials. Heart and kidney transplantation remains the only approach for end-stage organ failure. Allotransplantation of cadaveric organs, preparation of the donor and the recipient, and the surgical procedure are necessarily carried out under severe time pressure. Consequently, infections can be transmitted to the recipient (Morris et al. 2010). The average waiting time for a cadaveric kidney is five years, which significantly reduces the prospects for patients eventually receiving a donated kidney because graft survival drops substantially after extended dialysis. Although some guidelines have been developed to optimize the outcome of transplantations relying on deceased donors (Domínguez-Gil et al. 2011), tissues or organs are frequently taken from marginal donors, resulting in impaired graft function (Zimmerhackl et al. 2010).

The limitations of allotransplantation have prompted worldwide search for alternative treatments. Two different approaches offer potential solutions for solving the problem – the creation of human organs by blastocysts complementation and the transplantation of grafts from non-human donors. Both approaches provide the opportunity to closely examine donor tissue and thoroughly prepare the recipient before surgery. The strategy of growing organs in interspecies chimeras of pigs by complementing the blastocyst resulted in limited success, as grown organs can still cause immunological rejection in further transplantation to humans. It has become evident that the pig *(Sus scrofa)* is the donor of choice for xenotransplantation for several reasons: (i) similarities in size, anatomy, nutrition, and physiology as well as low genetic distance to man; (ii) short generation intervals (12 months) and high fertility (10–14 offspring per litter); (iii) well established and economic housing and breeding conditions at high hygienic standards; (iv) availability of advanced reproductive biotechnologies and genetic engineering techniques; (v) minor concerns regarding the slaughter of pigs, at least in western countries, because they are raised for meat production on an industrial scale; (vi) the risk of zoonosis is considered to be a minimal risk (strict monitoring, gnotobiotic environment), e.g. PERVs can be eliminated from the porcine genome and PERV-C free pigs are available. Therefore, the creation of GMO pigs for xenotransplantation is one of the promising ways to cope with the problem of organ shortage.

Despite these clear advantages, the selection of optimal immunosuppression therapy in each transplantation case needs further study. Moreover, the results of studies performed by different researchers is difficult to compare due to a large number of factors (organ type, pig used, etc.). In addition, there are other serious ethical concerns in society regarding the use of pigs as donors, and these need to be dispelled (Cozzi et al. 2009).

Nevertheless, recently, xenotransplantation has made great progress with the first heart transplanted from the genetically modified swine source that had 10 individual gene edits to a human in the US (Griffith et al. 2022). The recipient of the pig heart was in good condition after 5-week post-transplantation, which is longer than the survival of the first patient who received a cardiac allotransplant (Brink et al. 2017; Graham 2022). Unfortunately, sudden diastolic thickening and failure of the xenograft occurred on day 49 after transplantation, and life support was withdrawn on day 60. However, subsequent full investigitaion revealed that the patient's heart was affected by porcine cytomegalovirus, a preventable infection that is linked to devastating effects on transplants (Regalado 2022). Interestingly, this case emphasizes the need of considering swine genes responsible for the viral sensitivity to be also modified besides the other ones.

Additionally, genetically modified porcine kidneys have been connected to the blood system of two brain-dead patients. These experiments were terminated after 53 and 77h without any signs of rejection and fully functional kidneys with normal urine production (Porrett et al. 2022). These three experiments clearly show that xenotransplantation has started to enter the clinical stage and has the potential to overcome the shortfall of available human donor organs.

Conflict of interests

The authors declare no conflict of interests.

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