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Solving the global organ shortage by researching pig organ transplantation

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Abstract. Diseases such as organ failure or serious organ damage caused by accidents make organ transplantation become a key research area for scientists in today's society. The immune rejection and the lack of organ supply after organ transplantation are important factors limiting the success of transplantation. Due to the particularity of this field and the urgent need of researchers for clinical entity trials of organ transplantation, organ allotransplantation of non-primate, pigs, which are similar to humans in physiological and anatomical aspects and have high moral rationality, has attracted wide attention. With the in-depth research of immunology and genetics, gene coding, gene knockout and other technologies began to be applied to pig kidney and heart transplantation. Due to the obvious structural advantages of pigs, and the high familiarity of humans with pigs, the transplantation of highly complex lungs and livers can also be solved by genetic technology. From the perspective of individual tissues and genes, this paper explores the feasibility of pig organ transplantation by discussing the difficulties and solutions of pig organ transplantation, hoping to shed some light on the future development of xenotransplantation.

Keywords: xenotransplantation, immuological rejection, gene editing.

1. Introduction

Organ transplantation is the hope of life extension for patients at the final stage of organ failure, and millions of patients around the world have gained a new life through organ transplantation. However, in today's society, the demand for organs far exceeds the supply. In the early 20th century, the United States firstly completed a single organ transplant operation, and surgical science began to be used for organ transplantation, but it could not alleviate rejection and side effects due to technical reasons. In the 1970s, immunology began to be applied to organ transplantation in order to reduce rejection[1]. China completely stopped using organs from executed prisoners as a source of organ donors on January 1, 2015, making voluntary donation the only way to obtain organs for transplanting[2]. In China, only 0.67% of patients with organ failure can get an opportunity for transplanting every year.

Today, regenerative medicine has brought new ideas to organ transplantation. Autologous stem cells can reduce the rejection reaction to a large extent. The high reproduction rate of stem cells makes the obtained organs have a high probability of cancer, and the living environment of corresponding organs in vitro is difficult to be accurately controlled, and the human organs cultured in vitro are difficult to be used for transplantation. 3D printing technology has also been applied to organ reconstruction. However, due to the deliberate tissue structure of human organs and the complexity of

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blood vessels and nerves, the resolution of 3D printers is not enough to provide adequate tissue perfusion for the printed organs[1].

Due to the complex and fine structure of human organs, in vitro organ culture is limited by technology. With the deepening of biological science research, experimental animals have been widely used in human medical research and gradually applied in the field of allogeneic organ transplantation. In the early 20th century, animals were commonly used in clinical practice as donors to transplant tissue into human organs, especially heart valves[3]. In 2016, Baker's team explored the close relationship between kidney epithelial cells and human kidney failure by studying INK-ATTAC transgenic mice[4]. Animal tissues are extracellular products, transgenic mouse models are not directly related to humans, and the immune response caused by the both is weak. When the object of study becomes a solid organ, there are many factors that need to be considered during the research process.

At the end of the 20th century, in the field of xenotransplantation, patients with organ failure who received primate organs received prompt and complete immunosuppressive treatment, but they still died due to electrolyte disorders, complex infections or severe rejection after surgery. In contrast, non-primate pigs have greater advantages over non-human primates (NHP) as donors for allogeneic organ transplants. Pigs have organs similar in the size to those of adults, reproduce faster and are easier to be fed than mammals of the same size[3]. Pigs, as domestic animals, coexist with humans for a long time, with a lower probability of carrying unknown pathogens and higher safety. Non-human primates, which share the greatest genetic homology with humans, carry viruses that are easier to adapt to human cells, such as Ebola virus[5]. And the reproductive efficiency of non-human primates is low. Some large species are endangered, and the organ size of small species is too different from that of humans. All these factors make pigs the preferred choice of allogeneic organ donors in theory.

This paper explores the relationship between swine leukocyte antigen(SLA) and major histocompatibility complex(MHC). The role of gene knockout pigs (GTKO pigs) that knocked out α-1,3-galactosyltransferase (GGTA1) gene that promotes αGal synthesis in overcoming immune rejection has been discussed. The harm and weakness of endogenous retrovirus (PERV) in pig cells were analyzed. By analyzing the challenges and solutions faced by pig organ transplantation, the feasibility of pig organ transplantation is expounded, hoping to provide some inspiration for the future development of xenotransplantation field.

2. Kidney

Compared to chronic dialysis, kidney transplantation provides a better quality of life for patients with end-stage renal failure. The kidney structure of pig and human is smooth and multi-papillary, with similar cortex and medulla. Like human kidney, there are free anastomotic branches between the veins in pig kidney, which is conducive to the fusion of blood vessels after transplantation[6]. However, pig kidneys can react strongly to human growth hormone, and rejection can be reduced using growth hormone receptor knockout pig kidneys[7]. Swine leukocyte antigen(SLA) is an important immune response gene group in pigs, which plays an important role in the innate and adaptive immunity of pigs. It is the encoding product of major histocompatibility complex(MHC) gene. In renal cells, the decrease of SLA expression reduces the rejection intensity of renal tubular epithelial cells. Human tumor necrosis factor α (TNF-α) can enhance the expression of SLA I, while human interleukin-17 (IL-17) can inhibit this reaction and reduce the expression of SLA, thus weakening the rejection reaction of renal tubular epithelial cells. Therefore, IL-17 production can be stimulated to reduce the rejection reaction after kidney transplantation[8].

3. Heart

A pig heart is similar in size to a human heart and is suitable as a donor for a foreign organ. However, higher human blood pressure can stimulate the malignant growth of the foreign heart, resulting in maladaptive hypertrophy of the graft and early diastolic heart failure after transplantation. It has been studied that the occurrence of this condition can be avoided by knocking out the growth hormone receptor (GHR)[8]. Medical scientists at the University of Maryland knocked out three genes that may

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cause immune rejection in donor pigs, inserted six human genes that prevent blood clotting, knocked out some overgrowth genes, and transplanted genetically modified pig hearts into the chest of patients with advanced heart failure, significantly alleviating the host's immune response and reducing the negative effects of drug use, prolonging the patient's life[9].

4. Lung

Compared with kidneys and hearts, the lungs contain more and denser blood vessels, which are directly related to the body's blood circulation. At the same time, the gas-blood barrier formed by the capillaries of the lungs is more sensitive to pathogens, and the immune rejection is stronger. This makes the selection of exogenous lungs more difficult. Studies have shown that human blood contains antibodies that can bind to the α-1, 3-galactose (αGal) residue antigen expressed on the surface of pig cells, triggering severe hyperacute rejection. α-1,3-galactosyltransferase (GGTA1), which promotes αGal synthesis in knockout pigs, inhibits αGal production, and the lungs of the GTKO pigs are able to overcome hyperacute rejection[10]. The cross-species incompatibility between the donor transmembrane glycoprotein CD47, which is widely expressed in vivo, and the signal-regulating protein α (SIRP-α) in vivo can trigger phagocytosis of the recipient blood cells[11]. After transplantation of unmodified pig lungs into humans, the expression of pig CD47 protein on pulmonary vascular endothelial cells is not recognized as autologous material by human SIRP-α-directed macrophages, so the macrophages are activated to release immune factors and attack pig lung cells. Therefore, the transfer of human CD47 gene into pig lung cells can reduce immune rejection.

5. Liver

Liver transplantation is more complex and difficult. The liver is the main organ for the synthesis of clotting factors. After transplantation, the liver cannot recover the clotting function in time, and the clotting factors are reduced ,making the clotting function abnormal. Although in early studies, porcine hepatic sinusoidal endothelial cells and Kupffer cells were isolated from human platelets, resulting in a large decrease in platelets and coagulation dysfunction. However, through in vivo and in vitro studies, scientists have learned that coagulation dysfunction and platelet abnormalities are related to homologous glycoprotein receptor 1(ASGR1) involved in allogenic platelet phagocytosis. Therefore, the administration of GTKO pig combined with aminocaproic acid can effectively relieve and treat coagulation disorders[12]. The difference in quantity and activity of various proteins in pig liver can also be changed by modifying genes to reduce the difference between grafts and human internal organs. In addition, because liver cells have a strong regenerative capacity, transplantation of pig liver cells is also an option to save patients with liver failure. Pig liver cells are an unlimited alternative source, containing lower immunogenicity than humans and even preventing cellular and humoral immunity in the human body after transplantation. In one experiment, the scientists transplanted a portion of a liver from a GTKO pig into a non-human primate. The recipient kept the original liver, but only removed the original spleen and inserted the graft inside the splenic fosse. The recipient animals did not show serious rejection and survived longer[13]. This method is less invasive than traditional complete organ transplantation, making the effect of transgenic cells more significant, with lower postoperative morbidity and higher safety.

6. Challenge and possibility

6.1. Potential infection

Pigs have been used as livestock products for a long time, making humans more familiar with them. Pigs are the intermediate link in the transmission of some human pathogens, such as the H1N1 influenza virus. Pigs also play a key role in the parasite life cycle as an intermediate host for certain parasites, such as taenia suis, which uses pigs as an intermediate host and lives in the human small intestine as adult worms[14]. In addition, the endogenous retrovirus (PERV) embedded in all cells of

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pigs does not exhibit toxicity in pigs, but there is a high possibility that the virus will recombine with human endogenous retrovirus (HERV) to form a new type of virus. PERV is highly expressed in the kidney and is divided into three subtypes: PERV-A, PERV-B and PERV-C. Subtypes A and B can infect a wide range of human cells, while subtype C can only infect porcine cells. The A subtype with high replication capacity can be combined with the C subtype to form PERV-A /C recombinants (PERVs), which are highly toxic and more likely to infect human cells. Unfortunately, the widespread nature of subtypes A and B prevents them from being completely eliminated, so the risk of recombination can be reduced by selecting low-titer pigs and screening pigs without PERV-C subtypes. The best strategy to eliminate the risk of PERV transmission is to edit the genes of pigs. Scientists have developed a method to use cluster regularly interspaced short palindromic repeats, associated protein 9 (CRISPR-Cas9) RNA-directed nuclease to inactivate 62 gene replicators present in pig cell lines. The resulting PERV inactivated pig strain had a 1,000-fold reduced risk of toxic transmission to human cells[10].

6.2. Immune cell

After foreign organ transplantation, human natural killer cells (NKcells) lyse foreign pig cells either indirectly or through direct contact through antibody-dependent cell-mediated cytotoxic effects (ADCC). CD16 is a receptor for crystallizable fragments (Fc). In the process of ADCC, the Fc region of the foreign cell interacts with CD16 on NK cells in the human body, and NK cells then secrete perforin and granzyme to dissolve the foreign cell. The inhibitory receptors expressed by human NK cells can recognize human MHC molecules, and this recognition can inhibit NK cells from functioning. However, MHC molecules in pigs could not produce inhibitory signals and could not avoid the clearance of foreign pig cells by NK cells[9]. Insertion of human leukocyte antigen E (HLA-E) gene fragments into the donor pig MHC molecule can mitigate this rejection[10].

6.3. Rejection reaction

Although the introduction of GTKO pigs can greatly alleviate hyperacute rejection(HAR), overcoming acute humoral xenograft rejection(AHXR) remains a challenge to achieve high long-term survival. AHXR is a slow rejection reaction mediated by natural antibodies and complement system in human cells, which causes immune cells, such as phagocytes, to attack the graft. Macrophages activate cytotoxic T cells while secreting a series of inflammatory factors such as tumor necrosis factor to attack allogenic antigens. The rejection reaction caused by macrophages can be effectively controlled by inhibiting the activity of macrophages. It has been investigated that in the normal physiological environment of human, CD47 protein in endogenous cells avoids the clearance of the body's endogenous immune system by binding with the signal regulatory protein a(SIRPa) contained in macrophages. If pig cells can express CD47 gene, the transplanted organ can resist the attack of macrophages, thereby reducing the rejection of acute humoral xenotransplantation[15].

6.4. Other factors

From the physiological level, the anatomical structure of pigs is compatible with human organs, and humans have a deeper understanding of the structure of pigs, and the safety of operation can be guaranteed. From a moral perspective, humans are generally more accepting of pigs as experimental breeding objects and organ transplant donors than non-human primates, which are global livestock products. However, pig allogeneic organ transplantation also raises a series of ethical questions. Pigs have a sacred status in certain cultures, which makes the removal of organs from pigs unacceptable to those people[3]. Allogeneic organ transplantation relationships require a large number of experiments and harsh feeding conditions to ensure the rigor of the conclusions, which has raised doubts among those concerned about the welfare of experimental animals. In addition, the risk to humans of other unknown endogenous viruses that pigs carry which are not harmful to themselves needs further study.

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7. Conclusion

The familiarity of humans with pigs and the individual advantages of pigs make scientists study the allogeneic organ transplantation of pigs increasingly deeper. Stimulation of human interleukin-17 (IL-17) can inhibit the enhancement of TNF-α on SLAI expression in pigs, thereby reducing the effect intensity of SLAI and weakening the rejection reaction of kidney. Pig heart and human heart have a very high similarity in structure and shape, and the difficulty of transplantation is less, the hardness lies in the hypertrophy growth of the heart after transplantation. This reaction can be inhibited by gene editing or by knocking out the relevant receptor. Lungs, with their dense blood vessels and blood-air barrier that produces a strong immune response, and liver, which are involved in making and purifying blood, the transplantation of both are tougher. Studies have shown that CD47 plays an important role in immune rejection of lung allograft. When the human CD47 gene is transferred into the donor pig lung cells, the recipient will not recognize the allogeneic CD47 protein and cannot activate the rejection reaction. For livers with highly regenerative hepatocytes, in addition to suppressing rejection from the immunological point of view, transplantation of liver tissue while preserving the recipient liver is an effective idea to extend the survival time after transplantation.

Despite the landmark achievement in the field of pig organ transplantation, the rejection of certain cultures caused by this research, the harm to humans caused by the as yet unknown viruses carried in pigs, and the confusion of the animal welfare community about this field will become huge problems for the future development of this area.

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