Efficient and Specific Analysis of Complement Regulatory Proteins in Transgenic Animals on Xenotransplantation

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Abstract: With the continuous development of modern medicine, organ transplantation has increasingly become an important part of clinical medicine. Medical researchers have made new contributions to the ultra-acute rejection of xenotransplantation by the more mature genetic engineering technology of cell biology. Xenotransplantation faces many difficulties among which the immune rejection is the first and foremost problem that must be solved. According to the theory of xenograft immunology, using transgenic technology to create transgenic animals which could overcome hyperimmune rejection is another dedication of xenograft study. It can be predicted that transgenic animals have great prospects allowing xenografts to enter clinical practice. It's mounting recognized that the modification of transgenic animals might be directed to vascular endothelial cells and construct specific transcriptional regulatory elements in the founding of transgenic animal. This article will review recent studies on the theory of xenograft immunology and up-to date researches using transgenic animals to overcome immune rejection.

1. Current status of xenotransplantation immunology

The limitations of allogeneic research and the long-term shortage of transplanted organs make xenotransplantation valued again. Providing another solution for xenotransplantation provides a solution for clinical transplantation and is a hot spot in transplant surgery, Xenotransplantation becomes hot spot in transplant surgery. With the ceaselessly advancement of modern medicine, organ transplantation has become the most effective methods of treating end-stage organ failure and turned into an important part of clinical medicine. In the treatment of clinical allogeneic organ transplantation, the shortage of donor organs becomes roadblock due to successful surgeries, the development and application of immunosuppressive agents and the improvement of transplant success rate. The application of transgenic animals in xenotransplantation studies can not only help to understand the mechanism of xenograft rejection, but also solve the problem of xenograft disorders and donor organ lack [1]. Using specific transcriptional regulatory elements structuring by molecular biology techniques helps to obtain the highly expressed specific tissue related to complement regulatory proteins in transgenic animal vascular endothelial cells. Considering some factors like physiology, shape, size, feeding conditions and animal infectious diseases of heterogeneous organs, swine and primates are currently ideal sources of xenograft organs. Nevertheless, on account of the scarcity of the primate populations, it's untoward to popularize them in clinical practice even if they are so closed to humans in linage [2].

The first problem of xenotransplantation that must be must solved is immune rejection. There are multiple aspects such as heterologous antigens, antibodies, complement and vascular endothelial cells in xenogeneic rejection involves. The adjustment in different stages can inhibit the occurrence of rejection at disparate angles [3]. It's often seen in some researches that after target genes is integrated into genomes of a host animal, then the host animal appears to distortion, dysgenesis or other diseases, some even die. Eliminating ethical issues, the possibility of viral disease transmission and donor hormone disorders, many scientists predicted that xenotransplantation may be a way to address organ shortages if novel genetically modified animals can be controlled with the immune response such as HAR, complement response and $CD4^{+}T$.

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2. Strategies for transgenic animals to overcome immune rejection

The emergence of transgenic animals is the inevitable result of advances in embryonic technology and recombinant technology. There is currently no simple and clear definition of transgenic animals, often species that carry foreign genes and can be inherited and expressed. Transgenic animals often referred to as a species that carries a foreign gene which can be inherited and expressed. A transgene is a gene or genetic material that has been transferred naturally, or by any of a number of genetic engineering techniques from one organism to another. The introduction of a transgene has the potential to change the phenotype of an organism. In the study of Kurome M, certain genes can be transformed in vitro by some experimental methods and integrated into male nucleus of fertilized eggs for expression. In most situations, vascular endothelial cells in heterologous donors are the primary target organ of the recipient immune system attack which determines that the genetically modified targets of heterologous donors should target vascular endothelial cells [4]. However, due to the potential harm of marker genes, low random integration rate and poor stability of foreign genes and defective gene targeting technology, current transgenic methods including microinjection, nuclear transfer, sperm mediated can't be applied in preparation of transgenic animals.

Despite considered as ideal donors for xenotransplantation, compared with allogeneic transplantation, inter-ethnic transplantation of swine has a more severe and faster rejection which is pre-existing in human body and expressed as natural autoantibody IgM and complement mediated immune rejection against heterologous vascular endothelial cells. Most theories suggest that rejection of organ transplants in human body is caused by the binding of IgM, which recognizes the graft site, to donor vascular endothelial cells. Vascular anastomosis caused by acute rejection is able to result in loss of transplant organ function in a few minutes to several hours after surgery [5]. In acute rejection, main target cells are endothelial cells of donor organs. If they are capable of expressing human RNA, it may release a heterologous complement regulatory protein to protect the donor organ from endothelial cell damage. Avoiding lysis damage caused by complement activation can increase the survival rate of graft.

Complement activation is particularly important in rejection and endothelial cells are the first target of host immune response. Antibody conjugates stimulate the complement system and the coagulation cascade, causing graft hemorrhage and platelet thrombosis that disrupts endothelial function. Some trans-acting factors regulate the transcription and expression of structural genes through the positive and negative regulation of RNA polymerase activity which determines differences in different stages of growth in vitro and in vivo.

After transferring some target genes to host animal, tissue cells will not re-express the target genes which determine that differentiated tissue cells may not be the destination of targets genes. There may be no corresponding trans-acting factors in the same tissue cells of the host animal and the original animal [6]. With the development of germ cells, integrated foreign gene enters various tissue cells, then the expression of the foreign gene loses tissue specificity and the function of the gene in a particula tissue cannot be understand. Target gene integrated into the chromosome stably and efficiently and fertilized egg is transferred from the to the fallopian tube and uterus of the pseudopregnancy material where they develop into individuals who can transfer target genes to progenies – transgenic animals. Common methods of preparing transgenic animals are microscopic, transcriptional virus method and the embryonic stem cell method which are used commonly can modify the donor gene for different purposes. Currently, commonly used methods for preparing transgenic animals have a variety of options including particle bombardment, microinjection, induced pluripotent stem cells (iPS cells), spermatogonia stem cell approach and retroviral method. Modifying the donor gene for different purposes provides new means and methods for xenotransplantation research.

3. Physiological function and other obstacles

The somatic cell nuclear transfer technique is to conjugate exogenous target genes into an

animal body cell cultured in vitro and then obtain transgenes by transplanting somatic cell nuclear to enucleate oocyte. Among the xenografts, the activation of endothelial cells induced by the host immune system in the host immune system is unavoidable and the original regulatory pattern has changed. Some promoters are down-regulated by their affected activities affecting their regulated expression and anti-rejection inevitably.

Through in-depth study of human genome and heterologous antigens and genes, it is possible to eliminate major antigenic genes that block xenografts, genetically modify donor organs or produce human receptors for generating receptor chimeras. In recent years, use of sperm as a vector for foreign genes to create transgenic animals has progressed. After cultivating mature sperm with foreign genes，activate eggs by fertilization. During DNA synthesis, the foreign gene carried by the sperm is integrated into the chromosome to form a complete expression system, producing transgenic offspring [7]. The genetic modification of donor swine produces organs that are resistant to the immune system of recipients which can best meet human needs. Removing the causes of immune disorders, the success rate of organ transplantation no longer depends on immunosuppressive agents, recipient bone marrow transplantation and radiation-derivational immune tolerance.

The swine sperm cell viability of untreated and fully washed were 82.8% ± 1.5% and ± 1.4% and sperm cell viability abnormalities were 9.9% ± 0.6% and 10.3% ± 1%. After 2 hours of incubation, most of the sperm cells in each treatment group showed normal morphology and forward motion. There was no significant difference in sperm biosynthesis and sperm abnormality rate between pre-treatment group and post-treatment group. The table and figure show the effects of PAMAM-D / DNA complexes on the pig sperm cell activity.

Table 1.The effects of PAMAM-D / DNA complexes on pig sperm cell activity.

ratio of nitrogen to phosphorus	exogenous DNA amount（ng）			
	100	300	500	700
10：1	81.3	80.5	80.2	79.5
30：1	82.1	79.6	78.6	77.4
50：1	79.6	78.9	79.2	76.9
control group	83.5	79.3	80.4	78.2

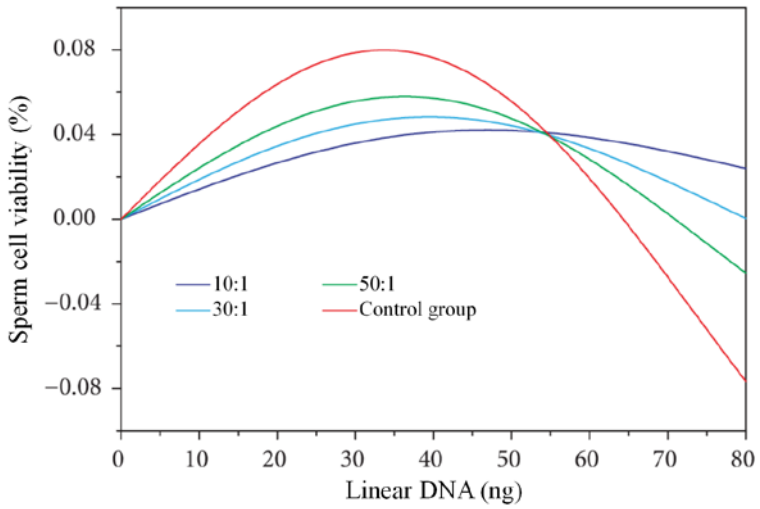

Figure 1. The effects of PAMAM-D / DNA complexes on pig sperm cell activity.

Acute vascular rejection, like immune rejection, is caused by anti-donor antibody binding to the

graft. Activation of endothelial cells by one or more mechanisms can result in releasing a small amount of complement or catalyzing the transplantation of endothelial cell dysfunction 8]. At present, scientists are using immunosuppressants, anti-endothelial activation and anti-thrombosis to prevent or treat acute vascular rejection [9]. Detection of transgenic animals mainly relies on molecular hybridization. The basic principle is to use molecular hybridization with a probe that binds to a target gene sequence to detect whether a foreign gene has been integrated into animal's genome. Another method of inhibiting expression in genetic engineering techniques is to use antisense RNA. According to the principle of sulfhydryl complementarity, inhibit or block the expression of particular gene without affecting the normal function of other genes by specific RNA that artificially or bio-specifically synthesized [10]. In addition to the host's anti-graft immune response, another question is whether the xenograft can provide adequate function to the host [11]. In the study of transplanting swine kidney and swine lung into primates, it can be seen that to a large extent, pig's organs are able to support receptors to maintain normal life function, but there are still some minor functional drawbacks that may need to be explained and solved through in-depth research.

4. Conclusion

Further understanding of the rejection mechanisms of xenotransplantation can provide a better strategy for addressing organ shortages and reducing the risk of organ transplant surgery. The ovarian injection method can integrate the xenograft gene into the sample body. Human serum pyrolysis experiments confirmed that spleen lymphocytes samples with three transgenic were more resistant to human serum lysis. Nevertheless, after injecting multiple genes simultaneously, gene integration and expression are much less efficient than single gene injections. So far, the method of microinjection using transgenic technology to inject a foreign DNA structure into the nucleus of a fertilized egg has a relatively low transfer efficiency. Nuclear transfer technology can selectively insert a target gene into a specific site and eliminate the function of the corresponding gene at that site, providing another effective means of producing transgenic animals. Persistent recognition of the mechanism of complement regulatory proteins in transgenic animals will open up new therapeutic strategies for medical development including xenotransplantation and is a medical development trend covering xenotransplantation. Applying multiple genetic strategies to modify donor organs, creating immune tolerance and inventing new immunosuppressants will allow xenografts to approach clinical practice.

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