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BYPASSING THE HLA SYSTEM IN ORGAN TRANSPLANTATION: OPPORTUNITIES TO ACHIEVE UNIVERSAL COMPATIBILITY VIA FULL SUPPRESSION AND ARTIFICIAL IMMUNOBIOCODING

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Abstract

In organ transplantation, the human leukocyte antigen (HLA) system is considered one of the main immunological barriers. HLA molecules are polymorphic antigens that serve as primary alloreactive triggers for the immune response. Incompatibility between donor and recipient HLAs increases the risk of graft rejection. Conventional approaches-such as compatibility matching, desensitization, or long-term immunosuppression-often provide limited efficacy. This study analyzes the potential of partially blocking the HLA system and forming a “Self” signal via artificial biocoding to restrict immune responses in transplantation.

Key words: Transplantation; HLA; immunosuppression; desensitization; biocode; CRISPR/Cas9; tissue compatibility; knockout.

Introduction

Using gene editing technologies (such as CRISPR/Cas9) and nanoimmunobiotechnological tools, it is possible to temporarily or permanently suppress HLA molecule expression, thus advancing the concept of universal donor compatibility. At the same time, molecular biocoding can potentially conceal the transplanted organ from immune detection in the recipient. These approaches aim to address donor matching limitations and significantly reduce rejection rates. According to data from the Global Observatory on Donation and Transplantation (GODT), more than 150,000 transplantations are performed worldwide each year. Organs and tissues are typically allocated based on criteria such as urgency, waiting time, histocompatibility, and HLA sensitization [1]. The introduction of universal approaches in transplantation—particularly the partial suppression of HLA or its substitution with an artificial biocode-

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offers a novel solution to long-standing challenges. This concept holds the potential to fundamentally reshape the practice of transplantation.

Main body

In the field of transplantation, particularly in the treatment of cancer and genetic diseases, modern engraftment methods such as cell therapy and hematopoietic stem cell transplantation (HSCT) are widely applied. However, these methods are often limited by alloimmune responses resulting from human leukocyte antigen (HLA) incompatibility. Successful HSCT requires the elimination of immune responses directed against donor or recipient HLAs. Suppressing the expression of the HLA-A gene using CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9) may be a promising approach for increasing HSCT efficacy by expanding the pool of unrelated donors. Allograft rejection is primarily associated with immune responses mediated by T and B lymphocytes against HLA molecules. The CRISPR-Cas9 system functions as a highly accurate gene-editing tool, enabling the cleavage or modification of targeted genes at the DNA level.

This technology is currently being used to selectively knock out HLA genes-especially HLA-A, HLA-B, HLA-C, and HLA-DR. When HLA genes are deleted from donor-induced pluripotent stem cell (iPSC) or embryonic stem cell (ESC) lines using CRISPR, the resulting tissues and organs are not recognized as “foreign” by recipient T cells. This offers a path toward realizing the concept of universal donors in transplantation.

According to scientific and clinical data, a 2019 study conducted by Xu H. and colleagues demonstrated that blocking HLA-A, HLA-B, and CIITA (a major regulator gene of MHC class II) via CRISPR enabled the generation of pluripotent cell lines completely lacking HLA class I and II molecules. These cells: were not recognized by T cells; retained HLA-E expression to reduce susceptibility to NK cells; and ensured long-term allograft survival without the need for immunosuppression [2].

Furthermore, in 2021, cardiomyocytes fully lacking HLA molecules were developed by deleting the beta-2 microglobulin (B2M) and CIITA genes using CRISPR. These cells were accepted without rejection and survived for extended periods in humanized mouse models [3].

From the perspective of biological safety, complete removal of the HLA system prevents recognition by T cells, but increases the risk of destruction by NK cells. To

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counter this, molecules that inhibit NK cell activity—such as HLA-E or HLA-G—can be preserved or artificially expressed, or the expression of immune-suppressive molecules such as PD-L1, CD47, and CD200 can be upregulated. These strategies not only reduce immune responses to the graft but also promote the induction of immunological tolerance.

Turning to the results, Kyoto University's Center for iPS Cell Research and Application (CiRA) is making consistent progress in developing an iPSC line bank for transplantation purposes. HLA-homozygous donors were selected, and 27 clinical-grade iPSC lines were generated from their peripheral or cord blood. Among them, 4 "super donor" lines were found to be compatible with approximately 40% of the Japanese population. Current reports indicate that the creation of 140 HLA-A, B, and DR homozygous lines may provide coverage for up to 90% of the population in Japan [4], and that CRISPR technology has been used to block genes such as HLA, B2M, and CIITA to create universal immuno-compatible iPSC lines that are now being tested in heart, kidney, and liver transplantation models [2].

Although HLA-deficient cells created through CRISPR represent a major achievement in preventing immune recognition, they are still vulnerable to natural killer (NK) cell attacks due to the absence of HLA molecules-referred to as the "Missing self" phenomenon. Therefore, to ensure real compatibility for transplantation, the concept of an artificial immunobiocode was developed. This approach enables the transplanted cell to evade immune surveillance while maintaining biological safety.

What is a biocode? It is a set of immunomodulatory molecules that either conceal the cell's presence from the immune system or emit a "self" signal. These include: Non-classical MHC-I molecules such as HLA-E and HLA-G, which bind to inhibitory receptors (CD94/NKG2A/B) on NK cells and suppress immune activation; Immune checkpoint molecules such as CD47 and PD-L1, whose expression protects the cell from phagocytosis and T-cell-mediated attack; Engineered β2-microglobulin–HLA-G fusion proteins, which act as surface polymers providing immunosuppressive signals [5][6].

In pluripotent stem cells, classical HLA-I molecules were eliminated by knocking out the B2M gene, and in their place, a β2m-HLA-E single-chain dimer was introduced [7]. This strategy protected the cells from NK cell "missing self" attacks and simultaneously prevented recognition by T lymphocytes [8].

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Through these approaches: T lymphocytes do not recognize classical HLA molecules, while NK cells interpret HLA-E/G signals as "Self," resulting in immunological tolerance, yet preserving the ability to detect infections or malignant transformations. Clinically, these immunobiocode technologies are currently being tested in SARs-T clinical trials, particularly in iPSC-derived heart, liver, and spinal cord tissues [9].

Such methods have demonstrated the potential to: reduce or even eliminate the need for immunosuppressive drugs; shorten transplant waiting lists; and ease donor matching constraints.

Feature	Stability in conventional transplantation	CRISPR-Based HLA Block + Biocode
HLA Compatibility Requirement	Strict matching required	Not required or significantly reduced
Risk of Allograft Rejection	High	Significantly lower
Immunosuppressive Drug Use	Long-term, lifelong	Minimal or possibly eliminated
NK Cell Activation Risk	Low (due to normal HLA expression)	High (HLA loss triggers "missing self")
Clinical Application Status	Established	Experimental (e.g., SARs-T trials)

Conclusion

Immunological incompatibilities associated with the HLA system remain one of the primary challenges in transplantation. Practical evidence has shown that editing HLA genes-particularly HLA-A or the entire HLA class I/II complexes-via CRISPR/Cas9 technology can significantly reduce the immunogenicity of donor cells. Additionally, artificial "immunobiocode" strategies involving the expression of molecules such as HLA-G, HLA-E, PD-L1, and CD47 have been shown to protect transplants from both innate and adaptive immune responses.

These technologies are increasingly recognized as promising approaches for implementing the concept of universal donors in transplantation, minimizing the need for

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immunosuppressive drugs, and overcoming limitations in donor matching. In the near future, large-scale clinical studies in this direction are expected to significantly enhance both the safety and efficacy of transplantation therapies.

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