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Review Article

CRISPR Mediated Genome Editing in Mammalian Stem Cells: Advances and Applications

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Abstract

Genome editing in mammals is becoming increasingly important due to its broad applications in agriculture, veterinary science, and human healthcare. Stem cells, including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult stem cells (ASCs), play a central role in this progress, offering valuable tools for understanding development, treating genetic disorders, and enhancing animal traits. The generation of iPSCs in various mammalian species offers an ethical alternative to ESCs, while adult stem cells, such as mesenchymal stem cells (MSCs), are widely utilized for tissue regeneration and improving livestock health. Although genome editing technologies like Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) hold significant potential, their use is still limited by technical issues such as low efficiency, off-target effects, mosaicism and the high cost of stem cell maintenance. Additionally, ethical concerns, animal welfare considerations, and stringent regulatory frameworks pose further obstacles. Despite these challenges, ongoing research continues to refine these methods, supporting their responsible application in both medicine and animal science.

Keywords: Genome Editing; Embryonic Stem Cells; Induced Pluripotent Stem Cells; Adult Stem Cells; CRISPR Cas9

Introduction

The importance of genome editing in mammals is growing due to rising global population pressures and the drive for economic development. It serves as crucial for improving food production by means of farm animals, advancing scientific research using mice as model organisms, and facilitating advances in genetic therapies and personalized medicine for humans. Consequently, modern research in agriculture and medicine has focused on more rapid and more accurate genetic improvement procedures [1]. Mammalian stem cell genome editing offers a potent platform for researching regenerative medicines, modeling genetic disorders, and studying early embryonic development. This makes it possible to improve characteristics linked to reproductive diseases resistance, and productivity in livestock for farming. It makes drug testing, cell-based therapy, and disease modeling easier in both humans and mice [2]. Stem cells, recognized for their remarkable capacity for self-renewal and differentiation, are the foundation of regenerative medicine and genetic research [3].

With the recent development of genome editing technology, stem cell genomes can now be altered with formerly unprecedented precision. Stem cells are typically divided into three categories based on their origin and characteristics: embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult stem cells [4]. Embryonic stem cells originate from the inner cell mass of a blastocyst, an early-stage preimplantation embryo. The ectoderm, mesoderm, and endoderm are the three germ layers into which these pluripotent cells can differentiate [5]. But using ESCs poses

ethical questions about killing embryos, which has sparked debates and resulted in regulations in several nations. To overcome this problem, certain transcription factors, including *OCT4, SOX2, KLF4*, and *c-MYC*, can be introduced into adult somatic cells to reprogram them into a pluripotent state, producing iPSCs [6]. A significant milestone in stem cell biology and regenerative medicine has been reached with the development of iPSCs. The development of iPSCs in humans, mice, and farm animals such as cattle [7], buffalo [8], goats [9], sheep [10], pigs [11], horses [12] and so on has been reported in several studies. Multiple insertion methods, such as lentiviral transduction, retroviral transduction, a transposon system, episomal plasmids, and transcription factor sets (*OCT4, SOX2, NANOG, LIN28, and C-MYC*), were used for developing these iPSCs [13].

Regenerative medicine has been revolutionized by iPSCs, which provide a supply of species-specific pluripotent cells while addressing ethical concerns related to ESCs [14]. Many organs and tissues contain adult stem cells, also called somatic or tissue-specific stem cells, which are essential for tissue maintenance and repair. Unlike ESCs and iPSCs, these adult stem cells are multipotent, meaning they can differentiate into a limited range of cell types related to their tissue of origin. Adult stem cells are widely used in animal science, with mesenchymal stem cells (MSCs) being especially important because of their many applications in enhancing livestock production and health, as well as their versatile regenerative abilities. These cells can develop into multiple cell types and are found in bone marrow, adipose tissue, and the umbilical cord [15]. This review emphasizes on advances in stem cell research and genome editing technology, enabling a better understanding of fundamental biological processes, improving reproduction, production, disease resistance, and disease modeling, and support the development of new treatments for degenerative and hereditary diseases [2] figure 1.

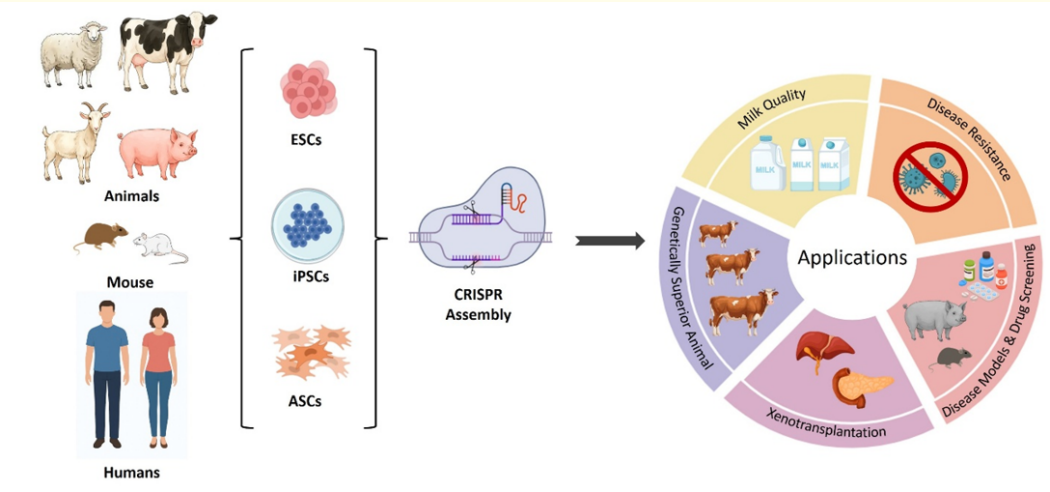

Figure 1: Application of genome-edited mammalian stem cells in regenerative medicine and animal sciences (ESCs: embryonic stem cells; iPSCs: induced pluripotent stem cells; ASCs: adult stem cells).

Genome editing in embryonic stem cells/embryonic stages

ESCs derived from fertilized eggs can be used to modify genes with CRISPR/Cas9 genome editing technology. However, the success rate of producing large-scale DNA knockouts through embryonic genome editing remains low, even though creating gene knockout mice with minor indel mutations is quite effective. Gene targeting using ESCs and generating chimeric mice via blastocyst injection continues to offer advantages over direct embryo editing, including high-throughput in vitro targeting and screening [16]. The viability of post-edited embryonic stem cells is further confirmed by larger model animals such as sheep, goats, cattle, and buffalo. These animals also demonstrate the editing efficiency of important alleles and the ability to achieve multiple gene edits in one procedure [17].

CRISPR-mediated mutations in murine and bovine ESCs targeting *POU5F1*, a homeodomain transcription factor, disrupt early embryo development and cell lineage specification. *POU5F1* mutations lead to the downregulation of *NANOG, GATA2,* and *GATA4*, thereby impairing blastocyst development. CRISPR-Cas9, using a single sgRNA, achieved an 86% knockout rate, with most embryos displaying mosaic bi-allelic mutations, leading to morula arrest and disrupted blastocyst formation, although *SOX2* expression remained unaffected [18]. Electroporating an RNP/CRISPR-Cas9 complex into bovine zygotes has proven to be an effective technique for genome editing. Dairy cattle with *MSTN* (myostatin) and *BLG* (beta-lactoglobulin) mutations, as well as beef cattle with mutations in the myostatin gene and *PRNP* (prion protein gene), have been produced and are valuable resources for future precise breeding [19]. Achieving targeted gene knock-ins in bovines remains challenging because the homologous recombination (HR) pathway is mostly inactive in the zygote before the first cell division. Introducing a gRNA/Cas9 ribonucleoprotein complex with a homologous-mediated end joining (HMEJ)-based donor template (with 1 kb homologous arms) targeting the H11 safe harbor locus increases knock-in efficiency in non-dividing cells. This HMEJ strategy outperforms HR, NHEJ, and MMEJ, achieving a knock-in rate of 5.1 kb for a bovine SRY-GFP template in *Bos taurus* ESCs [20].

In a caprine model, the fibroblast growth factor 5 (*FGF5*) gene was base-edited to introduce nonsense mutations, which prevented hair growth during the hair cycle. Base editor (BE3) mRNA and sgRNA, which cause nonsense mutations, were microinjected into ESCs. BE3 editing resulted in decreased *FGF5* expression, likely due to post-transcriptional regulation of *FGF5*; the design of the sgRNA was vital for targeting efficiency. This is similar to findings with ZFN or TALEN editing, where high mosaicism in microinjected ESCs has been linked to uneven mRNA distribution [21]. In ovine, at the one-cell stage after fertilization, the multiplex technique involving microinjecting multiple sgRNA and Cas9 mRNA was also used to create viable sheep with nonsense mutations introduced into the genes for *MSTN*, *ASIP* (agouti signaling protein), and *BCO2* (beta-carotene oxygenase), which are responsible for sheep muscle growth, coat color, and fat colour [22]. In porcine, multiplex gene editing via CRISPR/Cas9 demonstrated that IVF-derived embryonic stem cells could be treated with pooled gRNAs and Cas9 to target four genes simultaneously (*CMAH, GHR, GGTA1*, and *PDX1*), confirming the viability of multiplex gene knockout in a single step. In pigs, knockout has been performed to regulate organ size and the expression of pig-specific antigens, ultimately aiming to accomplish pig-to-human xenotransplantation. These targeted genes are essential for the formation and growth of the pig pancreas [23]. The CRISPR/Cas9 system, delivered into in vitro-produced porcine zygotes, efficiently induced mutations in *eGFP, CD163*, and *CD1D,* with 100% targeting efficiency at the blastocyst stage, although some embryotoxicity was observed. Using CRISPR with Cas9, deletions were induced in *CD163* or *CD1D,* and both genes could be disrupted simultaneously. Direct injection into zygotes resulted in piglets with mutations on both alleles [24].

Genome-edited farm animals have been created using cytoplasm microinjection or somatic cell nuclear transfer; however, these methods have many limitations that reduce their effectiveness. To deliver Cas9sgRNA ribonucleoproteins to bovine embryonic stem cells without harming embryo development, electroporation conditions need to be adjusted [25]. The future direction of

genome editing in stem cell biology, which could lead to a new era of personalized medicine and therapeutic options, will depend on maintaining a balance between innovation and accountability as research progresses.

Genome editing in induced pluripotent stem cells

Induced pluripotent stem cells have the potential to be valuable assets for disease research, tissue modeling, and regenerative medicine. Differentiating iPSCs in vitro has enabled the study of tissue diseases and developmental processes [26]. It may also facilitate preclinical testing of therapeutic drugs for both veterinary and human medicine. Using differentiated iPSC lines to simulate disease and conduct high-throughput screening of small molecules for their effects on disease development has been successful in iPSC research [27].

The use of iPSCs in aiding genome editing to treat diseases and injuries in animals is expanding and is likely to become part of veterinary practice in the future; however, research on specific pathologies is usually limited in farm animals [28]. Regenerative treatments for domestic animals may also serve as models for human diseases. To replicate the cellular phenotype of a specific genetic condition, iPSCs can be generated from the host species carrying key disease-causing gene mutations. These cells can then develop into a particular cell type that reflects the relevant pathophysiology. This stem cell-derived disease-in-a-dish model can be used to investigate disease processes and identify novel therapeutic targets and compounds. To create cell and animal models for future research on chromosomal translocation-related genetic diseases, infertility, and cancer, site-specific chromosomal translocation was introduced into iPSCs using CRISPR/Cas9 [29].

Studies on iPSCs in farm animals remain limited, despite significant progress in genome editing using iPSCs in human research. With a strong framework of CRISPR/Cas9-mediated genome editing, researchers have recently used iPSC technology along with CRISPR-Cas systems to develop several new and reliable disease models. They have also devised innovative approaches for cell transplantation and targeted cell therapy for various diseases, including thalassemia, hemophilia A, cystic fibrosis, sickle cell disease, duchenne muscular dystrophy, and hereditary deafness [30]. CRISPR/Cas9 has been used to repair a hemoglobin beta gene mutation in iPSC derived from a host with beta-thalassemia that had normal hemoglobin beta function [31]. Similarly, CRISPR/Cas9 was employed to correct the expression of the trinucleotide repeat (CAG) in the Huntington gene (HTT) in iPSC neurons generated from a Huntington's patient. The corrected cells then developed into synaptically active neurons [32]. Additionally, several iPSC-derived models of alloimmune bleeding disorders, acute myeloid leukemia, and hereditary persistence of fetal hemoglobin have been extensively created in the context of hematological diseases to facilitate detailed investigation of disease pathophysiology. Targeting diabetes-related genes with CRISPR/Cas9 technology in human iPSCs has shown promise as a way to gain insight into the genetic aspects of this disease [30].

Some CRISPR systems, including CRISPR/Cas12, which has been used for iPSCs produced from spinal muscular atrophy, are currently advancing in the iPSC field. Base editors have been widely used to accurately correct gene mutations linked to disease [33]. Given these facts, the primary goal of CRISPR technology and iPSCs is to establish universal donor iPSC banks based on phenotypic diversity, thereby expanding the application of iPSCs in regenerative medicine. Progress has been hindered by issues such as inefficient reprogramming, a scarcity of species-specific reprogramming factors, and limited resources for defining iPSCs in livestock. Although studies on human iPSCs serve as a standard, the lack of research on farm animals highlights the need for focused efforts to adapt these advanced methods to agricultural species, where they could enhance productivity, disease resistance, and genetic diversity [13].

Genome editing in adult stem cells

Studies on adult stem cells, such as mesenchymal stem cells (MSCs), neural stem cells (NSCs), and hematopoietic stem cells (HSCs), have advanced due to the ability to genetically modify the genomes of isolated cells or animal models. This reveals key mechanisms that control the self-renewal and differentiation of adult stem cells. In primary HSCs from mice, highly effective gene

disruption was achieved through plasmid- and virus-free delivery of guide RNAs into Cas9-expressing HSCs or Cas9-guide-RNA ribonucleoprotein (RNP) complexes in wild-type cells. These methods enabled quick assessment of how loss of genes like *EED, SUZ12*, and *DNMT3A* affects function [34]. These techniques will significantly expand the applications of CRISPR/Cas9 technology in both normal and diseased hematopoiesis. Use of CRISPR/Cas9 gene editing to modify immune cells such as B cells, macrophages, T cells, and hematopoietic stem cells (LSKs) in mice, for example, by changing CD40 expression in LSK cells using Cas9 RNPs. Unlike viral-based methods, RNP reduces the time and effort required, allowing gene editing in any mouse strain. *In vivo* RNP-based CRISPR/Cas9 editing of transplanted HSCs offers a promising approach to studying gene function in the mouse immune system [35]. Bone marrow stromal stem cells (BMSCs), a type of MSCs, is hard to keep as primary cells alive and growing in the laboratory for a long time. Thus, their modification in immortalized BMSCs (imBMSCs) using CRISPR/Cas9 to insert the SV40T gene into a safe spot in the mouse genome (called the Rosa26 locus), allowing the cells to keep dividing without losing their original properties. enables them to grow continuously. These imBMSCs is a useful tool for both basic research and developing new treatments in regenerative medicine [36].

A co-culture system using mesenchymal stromal cells (MSCs) has been developed to improve the transplantation outcomes of CRISPR-Cas9 gene-edited human hematopoietic stem and progenitor cells (GE-HSPCs). MSCs support HSPC expansion in vitro and promote engraftment in vivo by secreting hematopoietic supportive and anti-inflammatory factors. These factors enhance the expansion and clonogenic potential of GE-HSPCs by reducing proliferation arrest, apoptosis, and inflammation [37]. It has been reported that engineered high-fidelity Cas9 variants, like HiFi Cas9 with the p.R691A mutation, decrease off-target editing while maintaining strong on-target activity. This mutation helps improve precision in genome editing. HiFi Cas9 enables effective gene targeting in human CD34+ HSPCs and primary T cells at five therapeutic loci (HBB, IL2RG, CCR5, HEXB, TRAC) and efficiently corrects the p.E6V mutation associated with sickle cell disease in patient-derived HSPCs [38]. CRISPR allows precise modifications in neural stem cells (NSCs), enabling researchers to explore the genetic basis of neurodevelopment, brain diseases, and neurological disorders. Aging impairs NSCs' transition from quiescence to proliferation, leading to faulty regeneration and less neuron production. A scalable in vivo CRISPR-Cas9 method identified 24 gene knockouts that boost NSC activation and neuronal growth in aged brains. Notably, deleting *SLC2A4*, which encodes the GLUT4 glucose transporter, reactivates aged NSCs, and increased glucose absorption in aging NSCs may contribute to decreased activation in mice [39]. While CRISPR-based strategies using adult stem cells have shown great potential in mouse models, their application in adult stem cells of farm animals remains limited, highlighting the need for further research to adapt these findings for agricultural and veterinary applications.

Compared to somatic cells, stem cells are more resistant to the electrical impulses used during electroporation, making them less susceptible to injury or cell death [40]. They also exhibit greater membrane permeability, enhancing the uptake of foreign DNA and leading to more effective gene editing [41]. Additionally, stem cells are less likely to undergo senescence and epigenetic changes during in vitro culture, thereby maintaining their functionality and genetic integrity over longer periods [42]. These traits make stem cells a more reliable and efficient choice, especially for live genome-edited animals. Recently, stem cells have become more widely used for genome editing because somatic cell nuclear transfer (SCNT) faces challenges in reprogramming somatic cells, especially those from aged or specialized tissues. This often results in inefficient reprogramming and developmental abnormalities in embryos [43]. To address these issues, stem cells offer significant advantages over somatic cells in genome editing. Unlike somatic cells, stem cells are easily reprogrammable. They can self-renew and differentiate into various cell types, allowing the creation of a wide range of genetically modified tissues from a single edited cell [41]. Additionally, stem cells generally show higher editing efficiency due to their active DNA repair mechanisms, which reduce the risk of mosaicism. Bovine embryonic stem cells (bESCs) exhibit higher genome editing efficiency, greater proliferative capacity, and lead to better somatic cell nuclear transfer (SCNT) outcomes, most notably lower rates of pregnancy loss, compared to other donor cell sources [44] (Table 1).

Table 1: Advantages of using stem cells over somatic cells for genome editing.

Features	Stem cells	Somatic Cells
Self-renewal	Divide indefinitely, making them ideal for long-term modifications	Limited division capacity
Differentiation potential	Develop into various cell types, allowing for more versatile applications	Already specialized, limiting their use
Genome stability	Generally, more stable after editing, reducing the risks of mutations	More prone to DNA damage and reduced lifespan
Efficiency of editing	Higher efficiency due to their ability to proliferate and expand post-editing	Lower efficiency as they have limited replication
Therapeutic potential	Used for regenerative medicine and transplantation after editing	Mainly useful for correcting mutations in existing cells, but not for long-term therapies
Risk of mutations	Lower risk if handled properly	Higher risk due to accumulated mutations in older cells

Challenges in stem cells

Recent studies highlight CRISPR potential for editing stem cells, with valuable applications in human health, livestock enhancement, and veterinary care. However, its use in stem cells is restricted by ethical issues and technical challenges. Factors like long culture periods, high maintenance costs, low editing efficiency, the risk of mosaicism, and unintended genetic modifications make its application difficult in mammals [45]. While CRISPR offers promise for boosting disease resistance, growth, and productivity in animals, similar safety, long-term, and equitable concerns also exist in human health [46]. Additionally, widespread adoption of this technology is hindered by strict regulations, animal welfare issues, and the technical difficulties of achieving precise, efficient genome edits in real-world settings.

Conclusion

The integration of genome editing with stem cell research represents a major advancement in both the agricultural and medical fields. In livestock, it presents opportunities to improve breeding efficiency, enhance disease resistance, and support sustainable animal production. In human health, it offers potential for developing personalized treatments, regenerative therapies, and solutions for genetic disorders. However, the broader application of these technologies remains limited by ethical concerns, technical difficulties, and regulatory restrictions. Continued progress will depend on improving editing precision, gaining deeper insight into stem cell behavior, and ensuring safe and effective delivery methods. A balance between scientific progress, ethical considerations, and regulatory standards is needed to ensure responsible and significant outcomes for both human and animal health.

Conflict of Interest

The Authors have no conflict of interest.

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