

GENOME EDITING: THE CUTTING EDGE GENETIC TOOL IN POULTRY PRODUCTION

Abstract

Avian species are used as potent model systems in research in varied fields as cell biology, biotechnology, genetics etc. Chicken contributes significantly for the world's supply of animal protein and is considered as a valuable research model. Various genome editing techniques, especially CRISPR/Cas9 aids in the development and growth of biotechnological, agricultural as well as biomedical fields. Genome editing methodologies possess the potential to improve avian health and production. These genetic tools in poultry have drawn lot of attention due to the prospective for highly productive hens, disease-tolerant avian lines, and the development of excellent biological models. The chapter highlights the contemporary techniques of genome editing in chicken and its implications.

Keywords: Poultry, Chicken , Primordial germ cells, genome editing, CRISPR/Cas9

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I. INTRODUCTION

Genome editing (GE) also referred to as gene editing or genome engineering, is a more recent and precise method of genetic engineering that allows for the modification of particular target genes in an organism's genome[1]. Gene editing technology can be used to add, remove, and replace gene sequences that contain genetic information. Single genes or single nucleotide polymorphisms can be changed using gene editing without introducing new DNA into the animal's genome. Today, GE has been applied to a variety of species, including monkeys, rabbits, sheep, goats, cattle, pigs, poultry, zebrafish, mice etc. It can successfully prevent the gene chain effect and interspecies reproductive isolation. It offers tools at comparatively low costs for innovation in biomedicine, agriculture, industrial biotechnology, and other areas related to the bioeconomy, efficiently resolving time-consuming, expensive, and inefficient challenges. It includes applications including transgene stacking, gene rectification, targeted gene insertion, gene tagging, investigation of wider genomic regions, and gene knockout. Sequence-specific programmable nucleases, which effectively catalyse genome editing in a wide range of species, are currently the most widely used means of genome editing.

II. GENOME EDITING METHODS

Nuclease, an enzyme that cuts a particular DNA sequence in cells, is used in GE to delete, repair, or replace particular disease-related genes. They can be programmed to generate targeted double-strand DNA breaks (DSBs) in genomic DNA. Thus, the process of GE relies on DNA repair system that occurs when DSBs induced by sequence-specific nucleases in DNA occur. High precision and efficiency are their most notable qualities. Sequence-specific programmable nucleases, which effectively catalyse genome editing in a wide range of species, are currently the most widely used means of genome editing. These can be broadly divided into two groups: I. Protein-guided endonucleases such as the Zinc finger nucleases (ZFNs) and Transcriptional activator-like effectors nucleases (TALENs) and II. RNA-guided end nucleases such as the CRISPR [clustered regularly interspaced short palindrome repeats] /Cas (CRISPR-associated nuclease) system [2,3]. Proteins like ZFNs and TALENs have a module that can be modified to detect a particular DNA sequence and direct an additional, connected module to cut the DNA. A TAL effector derived from *Xanthomonas* and the same FokI endonuclease used in ZFN make up TALEN. The TAL effectors play a crucial role in the recognition of nucleotides, while TALEN also recognises and causes double-strand breaks of specific nucleotides. The FokI endonuclease, which is present in *Flavobacterium okeanokoites*, is responsible for the double-strand breaks, while ZFN is made up of zinc finger motifs that can attach to DNA nucleotides. Compared to the sequential cloning procedures required to create specific DNA-binding domains for TALENs, it is simpler to synthesise and clone custom guide RNAs for Cas9 recognition. The CRISPR/Cas9 system employs short non-coding RNAs to direct the Cas9 nuclease to a target location in the eukaryotic genome, where it then cleaves the double-stranded DNA target. The CRISPR/Cas9 system is based on the CRISPR-Cas adaptive immunity system found in a number of bacterial and archival species. Prokaryotic DNA elements as CRISPR and Cas9 play significant functions in the bacterial immune system. Bacteria in the natural world contain CRISPRs that are exactly like viral genomes. When a virus enters a cell, a CRISPR attaches to the viral RNA and uses the Cas9 protein to damage it. It is possible to perform GE of essentially any portion of the cell genome due to the precise nature of the generated

changes in the DNA sequence. The benefits of ZFN and TALEN technologies for targeted genomic editing include their high precision and efficiency, adaptability to a variety of animal species, and the absence of exogenous transgene insertion, even in genetically modified animals. GE can quickly and cheaply create targeted plasmids thanks to advancements in TALEN and CRISPR/Cas9 technologies. The need for cultivating embryonic stem in the process of producing genetically changed off springs is also eliminated by the direct injection of editors into zygotes.

III. CHICKEN AS POTENT MODELS FOR GENOME EDITING

When used as a classic model, the chicken offers unparalleled and priceless benefits for understanding biological processes and their significance. Customized GE in chickens will herald a new era of scientific advancement, advancing our understanding of avian biology in general as well as its application to agriculture. In avian species, GE has made it possible to precisely and effectively modify the avian genome in order to maximize the system's utility for business and research. It has a number of advantages that could be used to enhance poultry growth and production performance, including increased egg production, increased immunity and disease resistance in birds, production of leaner birds with little to no fat deposition in meat for superior nutritional profiles and enhancement of digestibility as well as overall growth. The chicken is a very productive source of protein for humans, so improving its economic characteristics through genome editing will be beneficial in supplying a cheap food source. Through GE, it would be possible to increase both the quantity and quality of the protein in chicken. Another significant agricultural use of CRISPR/Cas9-mediated gene editing in poultry species is to enhance the performance of chicken by promoting muscle growth. The Myostatin gene (MSTN), which inhibits the skeletal muscle growth and development is a popular target for research into increased growth. In poultry, targeting MSTN to reduce its inhibitory effects on muscle growth can improve the growing performance of the birds. The CRISPR/Cas9 GE technology will be used by researchers to build a productive bioreactor system.

IV. IMPLICATIONS OF GENOME EDITING IN CHICKEN EGG TECHNOLOGY

A variety of egg features, including nutrient content, allergenicity, and the creation of bio-functional materials, could be controlled by altering the genes that make egg proteins like ovalbumin and ovotransferrin, as well as other egg proteins. The ovalbumin and ovomucoid genes are disrupted by CRISPR/Cas9 technology, which has the capability to result in eggs with low allergenicity and a lowered immune response in people who are sensitive to egg whites. Therefore, by ensuring that chicken products are allergen-free, GE is anticipated to play a significant role in the alleviation of allergic reactions to chicken eggs in some individuals. This kind of development is crucial for both the pharmaceutical industry's production of vaccinations and the designing of safe food goods. Furthermore, using GE technology to precisely insert genes into endogenous egg protein coding regions would enable the mass manufacture of useful functional proteins. A new generation of genome-edited avian models, knock-in/out chicken including specialised chicken bioreactors, disease tolerant models and low-allergenicity eggs have been made possible because of advancements in employing PGCs. The creation of disease-resistant avian lines is another anticipated result of avian GE technologies.

V. GENOME EDITING IN BIOMEDICAL RESEARCH

The CRISPR approach enables us to comprehend how genes function and/or to change an animal's phenotype to meet a particular scientific or production goal. The use of genetically modified chickens in sectors like agriculture, manufacturing, biological research, and pharmaceuticals has a lot of potential. Target sequences carried by retrovirus vectors have historically been mostly employed to create transgenic chickens. TALEN-mediated homologous recombination successfully replaced the DDX4 gene in primordial germ cells, and chickens with altered genomes were created after transplanting these cells. Companies that generate antibodies can use CRISPR/Cas9-mediated methods to purify over expressed human antibodies from chicken and quail eggs to create recombinant proteins and vaccines. Additionally, the CRISPR/Cas9 system's use to produce particular antibodies from chicken eggs represents an affordable and stress-free means of manufacturing antibodies for therapeutic uses. The creation of tailored eggs for vaccine production is another area where the GE of poultry has the potential to have a significant impact. Eggs with increased virus yields for vaccine manufacture could be produced by editing chicken genes. The entire vaccine production process would be affected by the potential to manufacture high vaccine yield eggs. With the evolution of precision GE, it is now possible to insert particular marker genes on the Z chromosome, which determines male sex. When a female with a marker gene on her Z chromosome crosses with a wild-type male, the result is always males with the marker gene and females without it. Since significant success has been accomplished in chicken and quail, GE research will now be expanded to other poultry species around the world, including turkeys, geese, ducks, and guinea fowl [4].

VI. CRISPR IN CHICKEN

The leading-edge and most sophisticated gene-editing tool is CRISPR technology, which enables scientists to change and modify gene functions in the animal genome for gene targeting and therapy, epigenetic modification, for transcriptional regulation, gene targeting, regulation of transcription and drug delivery[5]. Only two types of poultry (chicken and quail) have seen significant advancements using CRISPR/Cas9 technology to date, with chicken making the most progress. CRISPR/Cas uses a guide RNA to instruct the Cas enzyme to cleave the cognate nucleotide sequence, an approach inspired by the adaptive defense mechanisms of bacteria and archaea. The most popular of these systems, CRISPR/Cas9 from *Streptococcus pyogenes*, typically uses a bipartite guide consisting of a CRISPR RNA (crRNA) that recognizes the roughly 20 nucleotide target site by Watson-Crick base pairing and a trans-activating CRISPR RNA (tracrRNA) that hybridizes with the cr RNA and complexes with the Cas9 nuclease. In comparison to ZFNs- and TALENs-based methods, the application of the CRISPR/Cas9 comprises of a number of benefits: it is much simpler to carry out, more effective, and appropriate for high-performance and multiplex GE in a varied cell lines and in living organisms. In the first chicken CRISPR/Cas9 study, in 2015, chicken embryos were electroporated with plasmids encoding Cas9 and guide RNAs.

VII. PRACTICAL APPLICATIONS OF CRISPR IN CHICKEN

Additionally, the introduction of CRISPR vectors into PGCs was successful in producing chickens with altered genomes that had substituted gene cassettes in the immunoglobulin gene and ovomucoid gene indel mutations. Erythrocyte selection combined

with HDR-CRISPR/Cas9 is a potent and quick technology for creating recombinant avian influenza vaccines. Furthermore, the erythrocyte-adsorption assay can be used with any target antigen that has erythrocyte adsorption activity and HDR-CRISPR/Cas9 can be used to create recombinant vaccines to protect chickens against a wide range of economically significant pathogens. The use of CRISPR/Cas9-based GE as a quick and effective way to create Herpes virus of turkey (HVT) recombinants that express the Infectious Bursal Disease virus VP2 protein. This strategy offers a productive way to incorporate additional viral antigens into the genome of HVT for the quick creation of recombinant vaccines. Additionally, it has been employed to study the interactions between viruses and their hosts through whole-genome screening and the identification of host factors necessary for viral replication. Under the control of single stranded RNA and the Cas9 protein, CRISPR/Cas9 nucleases could target specific DNA sequences and result in DSBs in genomes. As a result, indels or exogenous genes can easily penetrate chromosome DNA breaks, resulting in targeted transgenesis or mutagenesis. The CRISPR/Cas9 nuclease system has been extensively used in chicken somatic and embryonic cells. An enrichment system was implemented successfully to disrupt the DF-1, PPAR-, ATP5E, Pax7, and OVA genes in chicken somatic cells. It was possible to create chickens with specific heritable mutations in the OVA and ovomucoid using CRISPR/Cas9 nucleases, and the JH gene was specifically deleted in PGCs of chicken and gene-edited poultry. In 2020, chNHE1-KO homozygous mutant chickens that are resistant to Avian Leukosis virus infection have been produced by using CRISPR/Cas9 and PGCs technologies.

VIII. CHALLENGES OF PRECISION ENGINEERING IN CHICKEN

The CRISPR/Cas system uses a guide RNA to instruct the Cas enzyme to cleave the appropriate nucleotide sequence, drawing inspiration from the adaptive defence mechanisms of archaea and bacteria. The use of spermatozoa vectors in conjunction with CRISPR/Cas9 technology can produce transgenesis in the first generation, resulting in significant time and resource savings. CRISPR derived from the immune system of *Prevotella* and *Francisella* 1 (Cpf1) function similar to the CRISPR/Cas9 system. "Base editing," which could be used for gene correction devoid of homologous recombination, is the process by which CRISPR-cytidine deaminase complexes convert Cytidine into other nucleotides on targeted loci without indel mutations. The challenges of accessing the early avian zygote and failure to support the developing embryo post-injection/transfection prevent the transfer of this technology to avian embryos. Due to this, the majority of genome editing studies for birds that have been published till date have discussed the implementation of CRISPR/Cas9 in avian tissues and somatic cells. The CRISPR system is impacted by a variety of variables, including off-target effects, delivery methods besides the frequency of homology directed repair. Off-target effects may be crucial in identifying and eliminating hypervariable viral nucleic acids or the plasmid DNA of helpful bacteria that may change a bird's microbiome profiles. The low transfection efficiency (2 %) of avian cells in GE of the CRISPR/Cas9 system in poultry is another significant drawback. Additionally, germ-line transmission efficiency is only 10%, which is considerably low. We can make better use of this technique, as well as increase its specificity and efficiency, by addressing these potential pitfalls.

IX. CONCLUSION

Genome editing is used in animal husbandry to reduce the cost of breeding, study developmental biology, improve food production, control disease and vectors, combat antibiotic resistance, create disease models etc. Because of the potential for greatly productive chickens, disease-tolerant avian lines and the creation of exemplary biological models, GE in poultry has received substantial attention. The use of GE entails risks because it has the potential to cause damaging off-target mutations. Additionally, it might cleave unintended sequences that lead to mutations that lead to cell death or change. If these dangers are eliminated, it has a huge potential to enhance the economic characteristics of birds and, in addition, can produce disease-resistant birds that can promise poultry farmers additional rewards. The methodology of virus subgerminal injection could return to common use with the usage of adenoviral vectors for CRISPR/Cas9 delivery. The use of this technique might hasten avian knockout research and advance potential agricultural applications. In addition to these, it can be utilized to modify osteoporosis and heat tolerance in older laying hens. It is well known that this approach can be used to learn a great deal about genetics, gene function and genetic relationships. Through the use of CRISPR, it is possible to access genetic traits that would otherwise be inaccessible for the breeding of poultry. As a result, CRISPR/Cas9 emerges as a very potent and reliable tool for GE that enables the addition or control of genetic information in the poultry genome. Specific developments brought about by GE will pave the way for innovative ideas in disease control, welfare enhancement, food safety and vaccine production.

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