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Manoj Kumar
Animal Genetics Division, ICAR - Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Devender Choudhary
Department of Veterinary Public Health, College of Veterinary and Animal Science, Bikaner, Rajasthan, India

Shiv K Tyagi
Animal Genetics Division, ICAR - Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Munish Gangwar
Animal Genetics Division, ICAR - Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Govind Singh Dhakad
Department of Animal Genetics and Breeding, PGIVER, Jaipur, Rajasthan, India

Shobha Burdak
Department of Veterinary Pathology, College of Veterinary and Animal Science, Bikaner, Rajasthan, India

Kiran
Veterinary Officer, animal Husbandry Department, Govt. of Rajasthan, India

Corresponding Author
Manoj Kumar
Animal Genetics Division, ICAR - Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Different methodologies and applications of genome editing

Manoj Kumar, Devender Choudhary, Shiv K Tyagi, Munish Gangwar, Govind Singh Dhakad, Shobha Burdak and Kiran

Abstract
Genome editing is a potent technology that effectively modifies organisms' genomes to achieve targeted integration of exogenous genes and treatment of specified endogenous genes. The methods used to edit or modify the genome have developed from earlier attempts using nuclease technology, homing endonucleases, and specific chemical approaches. Meganuclease, transcription activator-like effector nucleases (TALENs), and zinc-finger nucleases (ZFNs) are examples of molecular methods that were initially developed as genome-editing tools. Due to their side effects on targets other than their intended ones, these early technologies have lesser specificity. The most recent innovation in form of CRISPR/Cas9 technology offers improved effectiveness, viability, and multi-role clinical application, which is more encouraging. The CRISPR/Cas9 system has become a potent tool for targeted mutagenesis in recent years, enabling single base substitution, multiplex gene editing, gene knockouts, and transcriptional modulation. It has become practical and advantageous to produce animals with better features using gene editing. Given the high effectiveness and low cost of genome editing tools, especially CRISPR/Cas9, it is likely that a significant number of genome edited livestock animals will be produced in the near future; therefore, it is essential to thoroughly assess the benefits they will bring to the livestock breeding industry.

Keywords: Genome editing, homing endonucleases, TALENs, ZFNs, CRISPR/Cas9, gene knockouts

1. Introduction

In order recognize the function of a gene, the gene is often inactivated by homologous recombination or by blocking its m-RNA by RNA interference. This is done in cultured cells through transfection or in living organisms by transgenes is (Feng, 2013) [7]. With advances in genome editing (GE) researchers can manipulate any gene at specified locus in a broad variety of species and tissues, including cultured cells and animal organs. Genome editing is a powerful technique for biomedical research and may be helpful for correcting the inherited diseases. The discovery of programmed sequence-specific nucleases (SSNs) for accurate GE is as a breakthrough in genome editing engineering. The SSNs are used to generate sorts of mutations as insertions, deletions, replacement, substitutions, addition of known sequence of DNA at a desired locus across many organisms and specific cell type. Though all kinds of SSNs have unique features with similarity of mechanism of producing double-strand breaks (DSBs) within the target DNA and These DSBs are re-joined via non-homologous end joining (NHEJ) or homology-directed recombination (HDR). NHEJ is an error-prone DNA repair mechanism that causes direct end-joining of DSBs without requiring a homologous template and may generate indels at target sites to develop gene knockouts. Additionally, NHEJ also can introduce insertions at the site of the DSB during operation of the repair mechanism. On the opposite hand, the HDR repair is more accurate mechanism that requires a homologous template to repair DSB and can be used to attain precise changes like gene insertion and gene replacement (Jinek 2012) [11], Kim 2014) [12]. These mutations can interrupt, eliminate or correct inherent errors in genes/DNA that cause diseases the defects in genes. The ‘programmable’ nucleases include mainly mega nucleases (Epinat, 2003) [6]. Zinc-finger nucleases (Urnov, 2010) [24], transcription activator-like effector nucleases (Miller, 2011) [18], and the CRISPR/Cas9 system [involving the Clustered Regularly Interspaced Short Palindromic Repeats and nuclease(s) associated to the CRISPR locus (Jinek, 2012) [11]. Usually, the CRISPR/Cas9 system is composed of a guide RNA (gRNA) that directs the Cas9 nuclease to make DSB at a specific site of the genome. Because of its simplicity, speed, and efficiency in genome editing in any cell or target tissue, CRISPR/Cas9 technology has

acquired general adoption over other techniques in the recent decade. ZFNs were first artificially engineered genome editing tools which composed of the binding module zinc finger protein (ZFP) with the nuclease domain FokI (an endogenous restrictive endonuclease from *Flavobacterium okeanokoites*). To edit the DNA/genome, a pair of ZFPs binds with regions flanking the target locus to make a FokI dimer, which is important to generate double-strand breaks (DSBs) (Kim, 1996) [13]. Like ZFNs, TALENs are also modular proteins with two domains: a TALE programmable DNA-binding domain and a FokI nuclease domain. Dimerized FokI produces DSBs by cutting TALE-binding DNA sequences. The scientific society selected TALEN-mediated gene editing as one of the top ten scientific discoveries in 2012, and both ZFNs and TALENs have been widely used in genome editing (Yang, 2011) [29] (Yao, 2016) [30]. The complexity of cloning and protein engineering ZFNs and TALENs has hampered their widespread adoption by the scientific community (Komor, 2017) [14]. CRISPR has changed the field in this regard because it's much simpler and more flexible to use.

Genome editing has been utilized in several sorts of biomedical research. Constructing an appropriate animal model of disease is important for studying the mechanism of human disease, and it also plays a crucial role in drug development and organ transplantation. In the field of gene therapy, genome editing is also a very useful technology. The programmed therapeutic elements have the potential to directly correct genetic mutations in targeted tissues and cells for treating diseases which are refractory to traditional therapies. In this review, we will discuss current advances in genome editing and its application animal science.

2. Different Methodology of Genome Editing

2.1. Meganulceases

MNs were the first class of nucleases to be widely used in SSNs. MNs were also named as homing endonucleases but in later they were used for generating DSBs. Meganulceases are the most efficient delivery method for all vectors, including plant RNA viruses, because they can detect target DNA sequences of roughly 12–40 kb but they are difficult to redesign for target sequences different than their natural ones (Marton, 2010) [17]. The biggest impediment to re-designing MNs is non-modular features of individual proteins.

2.2. Zinc-Finger Nucleases

ZFNs, were initially described in 1996 and termed as chimeric restriction enzymes. Chimeric restriction enzymes were created by combining the non-specific FokI with the DNA binding domains of two dissimilar ZFPs, according to this study. The ZFNs were created by combining chimeric proteins: one that cleaves DNA and the other that binds to it (DNA binding Doman). The DNA binding domain was made up of 3–6 Cys2H is 2ZFs, while the DNA cleavage domain provided a Fok1 restriction enzyme. FokI is a homodimeric restriction enzyme isolated from *Flavobacterium okeanokoites* that belongs to the type IIS class of restriction enzymes. In order to cut DNA, the FokI nuclease domain must dimerizes (Zhang, 2013) [32]. For active cleavage when binding to DNA, two ZFN monomers with FokI dimerization and C-terminal fusion are required. Each monomer containing 3–6 ZFs are able to detect target DNA with 9–18bp. Each monomer of ZFN dimerizes to execute the cleavage activity for targeted DNA by targeting the spacer region of 5–7bp found in the adjacent half-site. ZFNs are smaller than MNs (approximately 300 amino acids (AA) in one monomer and 600 AA in a pair of nucleases), allowing them to respond to a variety of delivery methods. ZFNs are currently not suggested in a number of situations because to their low target specificity, limited number of specified target domains, and huge number of non-targeted editing operations (Zhang, 2013) [32].

2.3. TALENs (Transcription activator-like effector nucleases)

When a harmful bacterium named Xanthomonas was researched for various plant kinds in 1989, TALENs were identified. Due to the formation of a new protein known as transcription activator-like effectors (TALEs), Xanthomonas causes uncontrolled plant cell growth. TALEs target specific DNA regions and dramatically influence gene expression (Pennisi, 2012) [21]. To obtain suitable TALENs for targeted GE, TALENs are altered by modifying the TALE repeating domains required for specific target identification and then sequentially connected to Fok1 nuclease. The TALENs that identify 12–21bp similarly to ZFNs, and a spacer region of 14–20bp is required for Fok1 dimerization with a pair of TALENs. The DNA binding domain has a repeated sequence of 33–34 amino acid that is highly conserved, with varied 12th and 13th amino acids. These two sites, known as the RVD (Repeat Changeable Deeside), are very variable and have a strong link with nucleotide recognition. The ability to construct unique DNA-binding domains by selecting a combination of repeat segments having the right RVDs enabled by the direct correlation between amino acid sequence and DNA recognition (Schmid, 2013) [23]. Another advantage of TALENs over other nucleases is their target specificity. In order to design TALEN monomers with more than a 30bp cleavage site, 15–20 RVDs are typically used. When compared to ZFNs, TALENs are less toxic and have more selective target locations (Mussolino, 2011) [20]. The only disadvantage of TALENs for use as a precise tool for GE is their huge size, which ranges from 950 to 1900 amino acid. TALENs are normally delivered to cells by direct DNA integration or by a construct-carrying TALEN-encoding unit being into the genome. Although TALENs are more widely used for targeted GE than ZFNs, they still require an efficient method of assembling tandem repeats for binding to the targeted DNA region. Furthermore, the repeated nature and large size of TALENs are significant obstacles to their proper distribution (Pennisi, 2012) [21].

2.4. CRISPR/Cas System

CRISPR/Cas is a latest ground-breaking technology that acts as an adaptive immune system in prokaryotes (Bacteria and Archaea), protecting them against invading organisms during phage infection through spacer acquisition, synthesis, and target destruction (Bortesi, 2015) [1]. To acquire immunity, short segments of invading DNA known as spacers are incorporated between two adjacent repeats at the proximal end of a CRISPR (Clustered regularly interspaced short palindromic repeats) locus. During subsequent interaction with invading DNA, the CRISPR arrays, including the spacers, are transcribed and processed into short interfering CRISPR RNAs (crRNAs), which combine with the trans activating CRISPR RNA (tracer RNA) to activate and direct the Cas nucleases. This nuclease cleaves DNA sequences at specific site known as protospacers in the invading DNA. The presence of a conserved protospacers-

adjacent motif (PAM) downstream of the target DNA is a requirement for cleavage. A ‘seed sequence’ which is approximately 12 bases upstream of the PAM, must match between the RNA and target DNA to provide specificity (Mojica, 2009) [19].

The RNA-guided DNA/RNA cleavage in CRISPR-Cas is achieved by an "effector complex" made up of an RNA guide termed CRISPR RNA (crRNA) and a group of Cas proteins (Class 1) or a single multi-domain Cas protein (Class 2). Based on the identification of the signature protein that cleaves the target nucleic acid, the each of two classes are further classified into three types and further in several subtypes based on the CRISPR-Cas locus architecture. There are two classes, six types, and 33 subtypes in the most recent classification (Makarova, 2020) [16].

Class 2 systems are generally simple to adopt and have emerged as strong tools for genome manipulation applications in both prokaryotic and eukaryotic cells due to their simplicity, in which a single Cas protein is required to perform targets binding and cleavage. In the last several years, Type II CRISPR-Cas9 has been widely used for genome editing in a variety of organisms, from bacteria to eukaryotic cells (Makarova, 2020) [16]. A short noncoding RNA called the trans-activating crRNA (tracrRNA) base pairs with the repetitive sequence in the crRNA to generate a unique dual RNA hybrid structure during the silencing process (Brooks, 2014) [2]. Cas9 is guided to cleave any DNA having a complementary 20-nucleotide (NT) target sequence and adjacent PAM by this dual-RNA guide (Cong, 2013) [4]. In type II systems, tracrRNA is essential for crRNA maturation (Wang, 2013) [25]. The technique is simplified by a chimeric crRNA that merges the crRNA and tracrRNA into a single RNA transcript while keeping full Cas9 sequence-specific DNA cleavage functionality. This simplified two-component CRISPR–Cas9 system may target almost any DNA sequence of interest in the genome and induce a site-specific blunt-ended double-strand break (DSB) by modifying the guide RNA sequence (spacer) within the crRNA. Cas9's DSB is then repaired by either error-prone NHEJ, which results in small random insertions and/or deletions (indels) at the cleavage site, or high-fidelity HDR, which results in precise genome modification at the DSB site using a homologous repair template.

Unlike traditional nuclease-mediated DNA editing approaches such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), CRISPR–Cas9 DNA recognition is determined by the 20-nt guide RNA sequence rather than the protein. This eliminates the requirement for time-consuming DNA-recognition domain protein engineering for each DNA target site to be modified, greatly increasing its application for large-scale genomic alteration or screening and gaining widespread acceptance among scientists.

3. Application of genome editing techniques

The CRISPR/Cas9 system possesses a remarkable therapeutic promise for the treatment of several diseases when the genetic source of malfunction is known, as well as for the study of these disorders using cell or animal models. Genome editing-based treatments have the potential to compensate for mutations or restore gene function. Different approaches to single nucleotide polymorphism (SNP) editing have been used, including as wiping out the disease-causing gene, providing a protective mutation, or including a therapeutic transgene. So, we are listing here some applications of genome editing technologies.

3.1. The first human-pig 'chimera': In order to create a hybrid chimaera, human and pig cells were combined. This resulted in the formation of organs that could be produced in an animal and transferred to humans. It is made by putting human stem cells inside of a pig embryo. After the stem cells had been incorporated, the pig embryo was placed inside of an implant, where they were allowed to develop for three to four weeks. The Salk team was able to remove particular genes from fertilised mouse egg cells using CRISPR genome editing techniques (Wu, 2017) [28].

3.2. CRISPR gene-editing tool to help turn immune cell against tumour: Immune cells have been genetically modified to have increased capacity for eliminating cancerous cells in mice. The cells were engineered to express chimeric antigen receptors (CARs), which allowed the cells to recognise and attack cancer cells that carried the associated antigen, on their surfaces. Immune cells that had been modified to trigger CARs using CRISPR were more effective in eliminating tumour cells than immune cells that had been modified using conventional methods (Gebler, 2016) [8].

3.3. CRISPR/Cas9 approach for cystic fibrosis treatment: A mutation in the gene that produces the cystic fibrosis trans membrane conductance regulator (CFTR) protein results in genetic disease CF. A thin mucus that lubricates and protects internal organs (like the lungs and pancreas) is produced by normal CFTR proteins, which act as channels to allow the passage of charged ions and water in and out of cells. Due to an imbalance in the passage of water and ions into and out of cells caused by the defective CFTR protein, cells produce thick mucus that obstructs airways and traps microorganisms. The defective CFTR gene's incorrect DNA sequence is replaced with the normal one using CRISPR-Cas9 technology [9].

3.4. Gene Editing Technique for Neurons Could Boost Research in Brain Diseases: The CRISPR-Cas9 genome editing technology has been hailed as a major advance in medical investigation. Its main limitation is that it cannot be used on cells that have stopped dividing, such as brain neurons. AAV, a moderate viral vector frequently used for gene transfer, has been combined with CRISPRCas9. When they administered the combination to mouse neurons, HDR evolved significantly. It also worked when it was tried on an elderly mice model of Alzheimer's [10].

4. Genome editing in livestock for breeding

4.1. Milk modification: In milk of goats, cows and other ruminants (normally absent in human milk) *β*-Lacto globulin (BLG) is a major whey protein that can cause allergy symptoms ranging from mild to life-threatening. Two frequently used techniques to lower the allergenic potential of BLG are heat processing and enzymatic hydrolysis, however both biochemical processes are expensive and may diminish milk's nutritional value by producing undesired by-products. BLG has been mutated in cattle using both ZFNs and TALENs, suggesting that genetic manipulation may be a more direct way to lower BLG levels in ruminants' milk. Cattle bearing the BLG mutation throughout the TALEN system had no mature BLG (Wei, 2018) [27]. In addition, the

human lactoferrin (hLF) gene was knocked in using TALENs in goats. Large-scale hLF expression and the absence of BLG in milk were discovered by phenotyping in the goats (Cui, 2015) [5]. Luo, *et al.* achieved high expression levels of human serum albumin in cow milk using the same technique (Luo, 2016) [15].

4.2. Meat production, composition and quality: A protein called myostatin (MSTN) is secreted in muscle tissues, and its main function is to limit muscular growth. Its natural mutation results in a double muscle characteristic that has been initially observed in cattle and thereafter in sheep, dogs, and humans. In 2015, a ZFN-mediated MSTN-mutation in Chinese Meishan pigs resulted in normal animals that had 100% more muscle mass and less fat gain than wild-type animals (Qian, 2015) [22]. Goats with disturbed MSTN also have increased body weight and greater muscle fibre size (Wang, 2018) [26]. The fat-1 gene, which changes n-6 polyunsaturated fatty acid (PUFA) to n-3 PUFA in goats, was also inserted into the MSTN locus. By reducing the ratio of n-6 PUFA to n-3 PUFA, which has been linked to a number of fatal diseases, the genetically engineered goats produced meat that was healthier and had improved muscle growth performance (Zhang, 2018) [31].

4.3. Disease resistance: The most economically destructive disease to impact industrial swine worldwide is porcine reproductive and respiratory syndrome (PRRS). Because of the virus's genetic variability, vaccines against the PRRS virus (PRRSV) offer inadequate swine protection. The cellular protein CD163, which belongs to the scavenger receptor cysteine-rich (SRCR) superfamily, has been identified as the PRRSV's cellular receptor, making it a suitable target to prevent PRRSV infection. Pigs deficient in CD163 were produced rapidly using the CRISPR-Cas9 technology. As a result of this, more CRISPR/Cas9 precision editing was carried out by either removing SRCR domain 5 or swapping it out with the human orthologous CD163 domain (Burkard, 2017) [3]. These investigations proved that the SRCR 5 domain served as the virus's interface.

5. Conclusion

Genome editing technology offers different techniques to alter, control, ascertain, and predict the genomes of large animals, potentially opening up unique biomedical and agricultural applications. The rapid advancement of large animal genome editing has led to the creation of numerous valuable animals for xenotransplantation, the agricultural industry, and the development of human disease models. The development of genetically modified animals, organs, and tissues for agriculture, regenerative medicine, and therapeutic applications will also be accelerated by further improving the current genome editing technology and the emergence of new tools for precise gene modification.

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