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Short Communication

Advancement of Genome Editing in Livestock

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Genome editing is an efficient tool that can significantly modify the genome of an organism to attain desired endogenous gene alteration and integrated exogenous gene insertion. Genetic engineering of livestock has proven difficult. The development of genetically modified livestock remained hindered due to the shortage of embryonic stem (ES) cells [1,2]. In the mouse, targeted gene deletion can be conducted in ES cells, and then the ES cells containing the desired genotypes can be implanted into recipient blastocysts to produce chimeric mice that can pass the genotype to the germline. As a result of the success of this technique, the mouse has become the primary model in biomedicine for elucidating the role of target genes [1,3,4].

In current history, 3 types of engineered endonucleases have emerged: Zinc Finger Nucleases (ZFNs), Transcription activator-like effector nucleases (TALENs), and the Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9) system [1]. In Xenopus oocytes, the first zinc finger (ZF) motif with specific DNA binding affinity was discovered as part of transcription factor IIIa. A larger DNA recognition domain can be constructed by combining multiple ZFs, which facilitates specificity and performance. Individual zinc fingers connect to each other in a mostly independent manner, with some interactions between adjacent fingers affecting base pair identification [5]. The technique was immediately applied to the development of GE livestock. Exogenous GFP gene was inactivated in somatic cells using ZFNs, and GFP knockout pigs were developed using SCNT as concrete evidence. Researchers were able to implement targeted modifications on both alleles in livestock due to ZFNs' strengthened genetic engineering effectiveness, which could drastically reduce the time it takes to develop and extend GE livestock models [6,7]. The scientists demonstrated the use of the CRISPR/Cas9 technique in genome editing in mammalian cells for the first time. The CRISPR/Cas9 gene-editing system, such as Cas9/gRNA Ribonucleoproteins (RNPs), was much easier to construct and use than the ZFN and TALEN techniques [2].

While Cas9 nuclease allows for multiplex gene editing, it needs relatively large constructs or concurrent delivery of multiple plasmids, which are both problematic for multiplex screens or in vivo applications. Cpf1, a type V CRISPR/Cas system, has recently been recognized as a Cas protein that can also be engineered to cleave targeted DNA sequences [8]. The CRISPR/Cas technology was quickly applied to the genomes of livestock, including pigs, cattle, goats, and sheep. One notable example is the genetic knockout of the CD163 receptor in pigs to make them immune to infection with the Porcine Reproductive and Respiratory Syndrome (PRRS) Virus (PRRSV) [9]. Myostatin gene (MSTN) is from a growth hormone family, a negative regulator that inhibits skeletal muscle growth. Under some conditions, the MSTN knockout results in increased skeletal muscle development, which could be beneficial for meat production [10]. ZFNs is used to knock out the gene encoding the bovine whey protein lactoglobulin, which is a major milk protein and a dominant allergen [11].

ZFNs, TALENs, and CRISPR/Cas (RNA-guided DNA endonucleases) have all proven to be useful molecular tools. The ability to build and research model organisms, including large animals, has greatly improved due to these new technologies [12]. Due to its physiological resemblance to humans, the domestic pig is especially promising in this regard. The various genome editing tools each have their own set of benefits and drawbacks, and the choice of one

technology over another seems to be based more on the experience of the particular researcher than on the limitations of one of these technologies.

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