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CRISPR-Cas9: Advancing Veterinary Science and Revolutionizing Animal Health

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Abstract:

Animal diseases are bound to threaten livestock production, animal welfare, and human health. The researchers involve attempts at controlling and restricting the spread of such diseases for safe food production for animal-origin products. Immunogenomics, a combination of transcriptomic and genomic data, shows promise in evaluating and boosting host resistance against diseases. The next-generation sequencing based technology is an advanced system where high throughput of information regarding the genomic and transcriptomic profiles is gained as a system of host-pathogen interaction functioning. Advanced editions in the field of genome editing such as insertion, deletion, or alteration of any genes have opened possibilities commercially that livestock can manage disease in food production. The momentum of CRISPR/Cas9 targeted genome editing as viable target applications in animal breeding against diseases continues to be gathering pace. Immunogenomics is still a mostly unexplored area, but rapidly gaining momentum, and yet the scope in technological advancement with respect to animal agriculture production.

What is CRISPR?

CRISPR, as pronounced "crisper", stands for "Clustered Regularly Interspaced Short Palindromic Repeats". Those DNA sequences in bacteria are a group of extremely small and defective DNA sequences called CRISPR, and they represent still the insertion and contagious nucleic acid residues of viruses who attacked bacteria eons ago. These DNA plugs serve as templates for the current generations of bacterial cells that recognize and destroy similar viruses. The CRISPR/Cas system is critical for microbial immunity, effectively defending against extrinsic phage or plasmid invasion via DNA or RNA cutting. Against that onslaught of foreign DNA, CRISPR-associated Cas9, an endonuclease, moves in against the invader's encroachment. In 2012, a paper proposed that the CRISPR/Cas9 system is a potent tool for sophisticated cutting and splicing gene sequences.

The CRISPR-Cas System:

The human immune system is a defensive mechanism against infections caused by viruses, bacteria, and other foreign objects. It has both non-specific innate immune system and an adaptive immune one involving antibodies and lymphocytes. Some viruses can infect bacteria, including bacteriophages, and the CRISPR-Cas system is the counterpart of an adaptive immune response for bacteria. On infecting their bacterial hosts, these viruses get the bacterial cells to degrade their viral DNA or RNA. The bacteria thus use the CRISPR locus, a section of the bacteria's own DNA, to store the data of viral infections, thus acting as a vaccination card that is continuously up to date.

CRISPR-Cas9: Mechanism and Applications:

CRISPR-Cas9 is an immune system type found in bacteria. Scientists have adapted its components into a biotechnology tool for DNA editing. A DNA-cutting enzyme, Cas9, in conjunction with a programmable RNA molecule, acts as guide RNA. CRISPR-Cas9 is further used to precisely target nearly any gene.

How it Works:

Step 1: Targeting

Scientists have developed a Cas9-guide RNA complex. It binds to DNA within a human cell. Cas9 recognizes and binds a three-nucleotide-long sequence termed PAM, which appears very often throughout the genome. The guide RNA, programmed like a GPS, binds to a specific 20-nucleotide-long sequence complementary to the target sequence. This allows Cas9 to bind to any part of the gene coding or regulatory sequence.

Step 2: Binding

Cas9 binds to a PAM motif in the genome, then unwinds the DNA double helix by means of a process called complementary base pairing, coupling up with the part of the gRNA-targeted DNA that has become unwound. This process is initiated because the Cas9 protein, in turn, unwinds the gRNA-targeted DNA, allowing Cas9 to dissociate from the DNA as a duplex and form a DNA-RNA helix with its complementary DNA sequence.

Step 3: Cleaving

Binding to the RNA-DNA duplex then activates the nuclease activity of Cas9, causing a double-stranded break across the 3-bp region adjacent to the PAM region. This binding event spurs DNA-cutting activity that introduces specific cuts at a point three nucleotides upstream, resulting in a double-stranded break.

Step 4: DNA Repair

Cells contain enzymes that repair double-strand DNA. The repair process is error-prone and will lead to mutations that may inactivate a gene. One out of many applications of the CRISPR-Cas9 technology

is to cleave DNA at exactly the right position.

Prospects and Challenges:

These large animal models are very important for studying pathogenesis and performing preclinical studies. However, conventional gene targeting methods are inefficient and have a potential effect of mosaicism. The introduction of new genome-editing technologies such as the CRISPR/Cas9 system offers improvements in this context. These notwithstanding, the promise of BEs in establishing large animal models showed that indeed most human genetic diseases emerge from point mutations. When added to somatic cell nuclear transfer and genome editing of SSCs, BEs present a more efficient way of making genome modifications in large animals. Gene therapies are being considered as treatments for inherited diseases, and genetically modified animal models might aid in the establishment of the efficacy and safety of these treatments.

- **Ethics Safety**

  The International Summit on Human Genome Editing has insisted on the salience of safety in germline genome editing for clinical reproductive purposes. Some would insist that germline editing cannot hold as much great promise as current technologies like preimplantation genetic diagnosis (PGD) and in vitro fertilization; nevertheless, some acknowledge that from time to time, it can meet needs that PGD cannot. The fear of many is that one case of germline editing, when used for therapeutic purposes, could pave the way for non-therapeutic enhancement. Others argue that genome editing in the context of curing genetic disease should be permitted when it proves effective and low risk.

- **Informed Consent**

  It is argued that it is impossible to obtain informed consent for germline therapy since the envisaged patients are the pre-embryo or future generations. This argument is counteracted on the ground that parents routinely make many decisions affecting future children, including ones just as complex as PGD with IVF. One of the main concerns among researchers and bioethicists is whether truly informed consent from prospective parents could possibly be obtained when the risks about germline therapy are not understood.

- **Justice and Equity**

  As with other new technologies, genome editing is feared it will only be accessible to those who can afford it, exacerbating existing disparities in health care and other interventions. They worry, for example, that such germline editing, if carried to extremes, could produce a two-class society of people based on the type of engineered genome.

- **Genome-Editing Research Involving Embryos**

  Stem cell research employing human embryos confronts moral and religious objections, while embryo-related research may not involve federal funds. Currently, NIH has no funding provided for

gene editing in human embryos. However, bioethics and research groups posit that it ought to be of note if it is not for reproductive purposes. Countries such as Sweden, Ukraine, and China allow human embryo genome editing, at least for research purposes.

Potentials and Prospects of CRISPR/Cas9 Technology in Livestock Production:

CRISPR/Cas9 is a gene editing technology that is gaining momentum owing to its advantages over traditional methods. Its use has included gene mutation correction in mouse models of human disease and human primary adult stem cells from monogenic hereditary defect patients. CRISPR/Cas9 can target regulatory noncoding regions in the genome. The genome-wide studies and disease resistance markers can also be studied. However, this set of technologies faces several challenges in developing sustainable strategies for disease control in humans, crops, fish, and livestock.

Benefits of CRISPR:

Targeted genetic technology is a genome-editing technique found highly relevant for human productivity, animal health, and animal welfare. It stands for accuracy, specificity, efficiency, and low cost. However, mastering this technology does take time, making sure guide RNA is specific for a target gene. CRISPR has proven to be an invaluable tool for research, giving rise to biotech start-ups aimed at treating diseases of humans and animals. In several countries, this CRISPR/Cas9 is utilized to knockout genes in livestock for enhanced muscle mass, disease resistance, and genetic improvement for welfare.

- **Improving productive traits**

  The first CRISPR-edited animals were born in the year 2014 using the Superfine Merino sheep as a genetic background. The project aimed to improve meat production by knocking out the MSTN gene in Superfine Merino embryos. This causes double muscle lambs with a faster growth rate and heavier body weight. The knockout lambs were 25% heavier still whilst having no effects in the quality of wool as compared to Merino lambs. The ground-breaking research claims that centuries of selective breeding practices can be replaced by just a few months' worth of CRISPR-editing.

- **Disease-resistant animals**

  Emerging diseases, such as African swine fever, can have enormous repercussions on the industry production and trade of live animals and their by-products, with effects on veterinary public health and animal welfare. CRISPR might pave the way for the generation of disease-resistant animals such as pigs resistant to porcine respiratory and reproductive syndrome virus (PRRSV), incurring significant economic damage in the pig industry. CRISPR genome-edited pigs were produced with the CD163 gene being disrupted and with the PRRSV infection rendered resistant. The Roslin Institute suggested gene editing in the RELA gene in domestic pigs to offer resilient disease resistance against the disease-causing virus. Also, pigs resistant to Coronavirus were produced through CRISPR, a proof for its capability as a new tool for infectious disease management in livestock.

- **Improving animal welfare**

  In recent years, efforts to improve animal treatment and show compassion have gained momentum, aiming to avoid unnecessary suffering, improve animal care, and improve animal husbandry conditions. Gene editing may revolutionize animal breeding and production by using welfare-enhanced animals, which have improved stress tolerance, better health, higher resilience, and lower levels of pain. Dehorning of calves, a routine procedure in most countries, is a significant welfare issue. The development of horn-free breeds has been a major topic in animal welfare and economics within the European Cooperation in Science and Technology (COST). Researchers used TALENs to introduce a causative celtic mutation into the genome of a horned cow, leading to the availability of a polled Holstein bull within the next decade. Genome editing sperm in the testis can give rise to knockout animals, and if realized in porcine species, it could lead to genetic mutilation of male pigs. Avoiding these practices in animal husbandry may encourage public support of genome-edited animals for food chain production.

- **Large animals' models in research and biomedicine**

  Genome-editing techniques are crucial for biomedicine and basic research, particularly in large animal models of surgery. These models allow for relationships between instances and responses to common questions, such as knockout and single-base-swap-re offset techniques. Biomedicine acknowledges genetic variations in human diseases like cancer, diabetes, heart pathologies, and neurological disorders. Veterinary practice has not yet utilized large animal models for human treatments, but more people are interested in using farm animals for preclinical studies. Pigs are the most widely used animal species in biomedicine and xenotransplantation, with CRISPR-based approaches suggesting they could end hyperacute rejection and enhance tolerance to graft acceptance in pig-to-human xenotransplantation.

- **No pests from livestock: gene drive challenge**

  Although Genetic engineering is fraught with a host of technical challenges, it is capable of driving genes at higher frequencies across populations than Mendelian inheritance. Gene drive promotes genetic polymorphisms, this enables the good population to mobilize desired edits. Thus, pest management options are evolving beyond chemical and pesticide approaches. However, suitability and optimal design strategies for optimal sgRNAs limit CRISPR-Cas-based efficacy concerning off-target. The experimental approaches for studying off-target edits stimulated by CRISPR systems can be classified into two types: cell-free in vitro methods and cell-based methods. Cell-free methods like CIRCLE-seq and SITE-seq have greater sensitivity but lower rates of positive validation because of chromatin contexts in the gDNA. There is no optimal method to comprehensively assess the off-targets associated with CRISPR-Cas.

CRISPR Modifications and Advancements:

Developed genome editing technologies based on CRISPR modify DNA with increased precision and flexibility. Researchers have created flexible variants of Cas9 proteins, nickase Cas9 (nCas9), and catalytically dead Cas9 (dCas9)-variants that can bind to nucleic acids without cleaving the DNA strand. In contrast with Cas9s, the base editors carry out very precise nucleotide substitutions without creating a double-stranded break in DNA. Prime Editing is a combination of nCas9 with engineered reverse transcriptase and prime editing guide RNA (pegRNA) that are expected to be able to correct 89% of genetic mutations in humans. In addition to this, an RNA editing system has also been successfully designed that does not modify the DNA and is considered to possess a safer therapeutic profile. The CRISPR enzyme Cas12f from Axidibacillus sulfuroxidans is small; it has been re-engineered to deliver modified AsCas12f to Mice through AAV for efficient CRISPR-based genetic therapies. These engineered CRISPR systems represent a significant leap forward in the precision genome editing capacity as they allow for more specific on-target edits to be made over a wider range of sites and for broader delivery options to be taken.

Summary of CRISPR-Cas systems:

CRISPR-Cas9 technology has changed the face of genetic engineering, wherein it becomes useful in animal models in precise genome editing for rapid, low-cost constructions of disease models to study gene function, conduct gene therapy, translation research, and investigate species diversity. CRISPR allows scientists to change an animal's DNA sequence directly, which is quicker and cheaper than doing it the old-fashioned way. These models will allow researchers to study gene function in diseases, perform drug testing, and assess new therapeutic strategies. CRISPR-generated animal models bridge the gap between basic science and clinical application, allowing for accurate Pseudo-reproduction of human diseases; however, ethical concern and careful regulation remain essential to safeguarding animal welfare in animal studies.