Genome Editing with CRISPR/Cas9 System: Potential and Limitations

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The CRISPR/Cas9 method has gained considerable popularity in the recent years, and it has been recognized as a compelling tool for current modern medicine. CRISPR/Cas9 based genome editing is an indispensable tool in plant genetics as well as in breeding. The CRISPR/Cas9 system has been successfully applied in various plant species. It enables desirable crop traits by introducing DNA from nature-generated genetic variations within the crop itself, and not from another reproductively incompatible organism. The use of CRISPR/Cas9 avoids the need for protein engineering to develop a site-specific nuclease against a specific DNA target sequence, requiring only the synthesis of a new piece of RNA. However, the simplicity and specificity with which CRISPR/Cas9 can edit DNA is changing the pace of biological research in many areas.

Since the discovery of DNA double helix, many more new technologies are being developed to expand the CRISPR toolbox such as base and prime editing along with determining, analysing and altering genome sequences and gene expression patterns in cells and organisms. The ability to diagnose genetic diseases has developed rapidly with reductions in genome sequencing costs, extensive comparative analyses of human genome sequences and applications of high-throughput genomic screening. CRISPR/Cas9 holds immense potential as a therapeutic for diverse diseases in humans related to genetic disorders such as β-thalassaemia, tyrosinemia, and cancers. Till date, this technology has already demonstrated many potential applications to human illnesses including genetic disorders, tumours, and infectious viruses. CRISPR/Cas9 has also revolutionized the generation of transgenic animals as it has demonstrated an extraordinary efficiency, multiplexability, and ease of use, and enabling the production of animals with more extensive genetic modifications.

Potential of CRISPR/Cas9 in Molecular Medicine

CRISPR/Cas9 systems for functional genomic screening is expected to be used to explore the molecular mechanisms of a variety of cellular functions. This will allow rapid drug identification in reference to their therapeutic efficacy and also allow one to use the potential of personalized medicine by combining genomics, therapeutic targets and disease phenotype. In addition, the use of CRISPR/Cas9 as an antiviral strategy is a promising prospect. At the target sequence, the requirement of a protospacer-adjacent motif (PAM) site limits the application of Cas9 because canonical spCas9 only recognizes the NGG PAM site once every 8–16bp. A multiple PAM site selection system is required to increase the scope of the target. On the other hand, in general, the application of CRISPR technology in tumour immunotherapy may improve the effect of therapy and the application scope of tumour immunotherapy. The CRISPR system may also serve as a powerful tool for epigenetic studies, allowing for targeted manipulation of epigenetic markers, which will further interrogate epigenetic and transcriptional control relationships.

CRISPR/Cas9 in Plant Biotechnology

In plants, genome editing technology is generally based on type II CRISPR/Cas9 system which depends upon Agrobacterium tumefaciens or direct gene transfer, using cultured plant tissues. However, genetic transformation and/or regeneration from tissue culture is not efficient in many crops or other plant species because it takes a long time for selection and characterization of mutants and can generate somaclonal variation, which creates additional mutations. Therefore, to overcome this problem, the use of tissue culture-free genome editing systems, such as ribonucleoproteins (RNPs), viral delivery, and nano-particle systems provide efficient alternatives that can accelerate the genome editing process. Tissue

culture-free genome editing systems are likely to be simpler, cheaper, and less expertise-intensive. At the same time, it increases the efficiency of CRISPR/Cas9 and reduces the time required to generate edited plants.

More prominent strategies and systems to inhibit endogenous non-homologous end joining (NHEJ) activity are required, as there is a need to increase the efficiency of knock-in homologous recombination and this needs immense effort to improve the homology-directed repair (HDR) and viral vector efficiencies. Transgenerational CRISPR/Cas9 gene editing in plants is an advanced innovative technique in agricultural crop development or crop refinement. Thus, so far, CRISPR/Cas9 system with nanotechnology has been used to improve the quality and yield many valuable crops for future benefits.

Enormous progress has been made in addressing the challenges of standard gene therapy by developing new systems for precise modification of the genome. This has also helped to overcome some of the obstacles that have tormented the field of gene therapy for decades. In this reference, rapid advances are being made for increasing the specificity of genome-editing tools and increasing the sensitivity of methods for assessing this specificity genome-wide.

Limitations in CRISPR/Cas9 System

Despite the success of CRISPR/Cas9, the technique is far from refined. In certain situations, the editing process can result in off-target DNA being changed, causing unwanted effects. Also, CRISPR/Cas9 is a large molecular complex, with both the Cas9 nuclease and an engineered single-guide RNA (sgRNA) that helps the nuclease locate its target. This makes its delivery into the nucleus of the cell, where CRISPR needs to access DNA, unfavourable.

Effect on Efficacy- There are several factors that influence the efficacy of CRISPR-Cas9 system, especially if it is to be used for in vivo human gene therapy. These factors include target DNA site selection, sgRNA design, off-target cutting, incidence/efficiency of HDR vs NHEJ, Cas9 activity, and the method of delivery. Polymorphisms in the protospacer binding site may also reduce the editing efficiency.

Off-target Effects- One of the shortcomings of this current system is that it may exert off-target effects. Many studies have documented that, unintended cleavage and mutations were observed at untargeted genomic sites showing a similar but not an identical sequence compared to the target site. This has led to undesirable mutations (translocations, inversions, large deletions and insertions) resulting from the complex endogenous pathways that repair the double-stranded DNA breaks induced by Cas nucleases in the genome.

Toxic Effects- Due to the unique nature of the prokaryotic genetic profiles, the CRISPR/Cas9 system shows toxicity in a large number of microorganisms, which can easily lead to fatal chromosome breaks, which further result in low transformation efficiency and failure of gene editing. When the CRISPR components are encapsulated inside the nano-vectors, it partially shields their recognition by the host immune system and mostly reduces the generation of immune responses. Therefore, the potential of non-viral vectors to trigger an immune response should be carefully studied when designing non-viral CRISPR-Cas9 delivery systems. The immunogenicity of Cas9 nucleases and carcinogenic effects of CRISPR components, hence require exhaustive analysis and scientific explanations.

Large Size of CRISPR/Cas System- The large size of CRISPR/Cas9 system hinders the editing efficiency, and it is not suitable for packing into viral vectors for delivery to somatic tissues. A smaller-sized CRISPR/Cas is required for efficient genome editing of plants.

Mutations at Non-specific Loci- Mutation which are introduced at non-specific loci with similar, but non-identical, homology to the target sites is one of the most important complications of this system. It is difficult to identify them and require scanning of the genome for mutations at sites with sequence similarity to the gRNA target sequence.

Transgene-free Genome Edited Plants- In most cases of plant genetic engineering, foreign genes are introduced into plants and firmly integrated into the plant genome via Agrobacterium-mediated transformation. The presence of foreign gene in chromosome raises another important issue such as genetically modified organisms (GMO). After genome editing, the CRISPR/Cas9 and sgRNA construct is no longer needed and can be segregated away; the resulting transgene-free edited

crop plants would then be indistinguishable from natural variants. In fact, genome-edited crops are not considered GMO in several countries and are thus cultivated without the typical restrictions associated with GMOs. For this reason, transgene-free genome-edited plants are mainly obtained through laborious and time-consuming genetic segregation, which can be especially challenging for crops with large polyploid genomes.

There is also another aspect which needs to be considered when this technology is required to be commercialized in various countries. Owing to unavailability of proper understanding about pesticides, fertilizers, and other products developed by nanotechnology; therefore, it is necessary to upgrade the knowledge of cultivars so that they can grow cost-effective better and transgenic crops.

In nutshell, this specific technology has turned out to provide life sciences with a highly demanded tool which exceeds current gene-editing technology in affordability, efficiency, scalability, precision as well as programmability. As a result, CRISPR/Cas9 has seen a quick adoption in research programmes. Despite the limitations, this dramatically simplifies and greatly reduces the time needed for gene editing design and implementation. Therefore, the CRISPR/Cas9 system has been successfully used to edit the genomes of a broad range of species, such as Caenorhabditis elegans, Drosophila, Zebrafish, Bombyx mori and humans. The CRISPR/Cas9 technology as a powerful, inexpensive and quick to design genome editing tool has been applied in many fields, ranging from basic biology to cancer therapy. CRISPR/Cas9 can be a highly useful tool for editing genes and to potentially treat complex diseases. CRISPR/Cas9 technology has also been used to optimize the shape and size of the crops based on the consumer preferences. Several genes/quantitative trait loci responsible for crop appearance quality have been proposed. However, it still needs to be refined as a technique and therefore has made researchers to strive for improvements in this area, to make the process more precise and effective.

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