Genome Editing & Future

Introduction

Ever since the discovery of the DNA, several technologies have evolved to genetically manipulate the organisms for better agricultural and industrial applications. The next generation sequencing techniques have made the identification of the different genes and mutations in DNA easier. The GMOs and cloning techniques which are in use for a significant time now are the direct result of the advancement in this domain. These cloning techniques have been exploited for a wide array of medical, agricultural, industrial and research applications for the past several decades. However, despite all the development and progress there is a constant need for more advanced gene editing techniques that are precise and accurate. The use of programmable nucleases for genetic modifications has increased in the past decade for its potential to treat various inheritable diseases and cancer. In past decade three major classes of programmable nucleases have come to light– the zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and the clustered regularly interspaced short palindromic repeats (CRISPR)–associated Cas9 (CRISPR/Cas9).

Zinc finger nucleases (ZFNs)

ZFNs was the earliest developed genome editing tool with greater precision for their ability to alter the genetic content. The ZFNs are made of two domains, repeated zinc finger proteins (ZFPs) with their DNA-binding domains fused to the non-specific DNA cleavage domain of a FokI restriction endonuclease. ZFNs function as a dimer where each monomeric unit has an ability to recognize nine to 18 base pairs (bps) of DNA via the zinc-finger DNA-binding domain. There is a wide range of Zinc finger domains which recognize the distinct DNA triplets. These can be joined together to generate polydactyl zinc-finger proteins that can target a wide range of possible DNA sequences. However, the off target editing/mutations are the major concern associated with the ZFNs based gene editing. Therefore, there is ample scope for enhancing the efficiency of this process.

Transcription activator-like effector nucleases (TALENs)

TALENs has emerged as an alternative to ZFNs for genome editing. Like the ZFNs, TALENs comprise two programmable DNA-binding domains fused to a FokI endonuclease domain and works by introducing the double-strand breaks (DSBs). Transcription activator-like effectors (TALEs) are naturally secreted by the Xanthomonas spp. of bacteria which binds to the DNA of the host organism. The TALE DNA binding domain is multiple repeats having 33-35 amino acids where each repeat recognizes a single nucleotide. The specificity in the TALE DNA binding domain is due to the presence of hyper variable regions present in each domain at 12 and 13 amino acid positions. Therefore, the sequence specific TALENs can be generated by modifying these amino acid residues in hyper variable regions and linking the different TALE repeats together.

Clustered regularly interspaced short palindromic repeats (CRISPR)–associated Cas9 (CRISPR/Cas9)

In addition to ZFNs and TALENs, the CRISPR/Cas9 is another rapidly emerging gene editing tool. It is a naturally occurring bacterial system which acts as a component of bacterial adaptive immunity. It provides protection to bacteria from invading viruses and plasmids via RNA-guided DNA cleavage by Cas protein. There are three main components of the CRISPR/ Cas9 system–the CRISPR RNA (crRNA), the trans-

activating crRNA (tracrRNA) and the Cas9 enzyme. The crRNA is transcribed from the invading system and known as protospacer sequence and hybridizes with the tracrRNA that triggers and acts as base for the binding of Cas9 nuclease to the site of DNA cleavage. Unlike ZFNs and TALENs systems that recognize the specific sequences in the given genome sequence using different recognition proteins, the CRISPR/Cas system uses the RNA sequence complementary to the target genomic sequence. The advantage of the RNA hybridization in site specific cleavage triggers the use of chimeric "guide" RNA (gRNA) for the genomic editing, which is created by fusing together the crRNA and tracrRNA. The gRNA contains 20 nucleotide guide sequences designed for binding to specific DNA sequences. Therefore, the gRNA and Cas9 can scan the appropriate site and bind to it for creating site specific double strand breaks (DSB). Thus, the CRISPR/Cas9 system offers a relatively more accurate technique of gene editing as compared to the previous techniques and can be used for a wide range of applications.

Applications of Genome Editing Technology

The genome editing techniques can be widely employed for treating the various inheritable disorders. The genome editing system has been successfully used in editing genes of the human cells taken from the patients suffering from the different inheritable disorders. Other than therapeutic genome editing, the genome editing techniques can be widely used for synthetic biology and genome scale engineering. For instance, for altering or mutating the genome of bacteria or yeast to produce industrially important secondary metabolites; or for modifying the biological strains for enhanced efficiency in any biologically important conversion reaction.

The genome editing tools can be very efficiently employed for targeted gene regulation as well. For instance, accelerating wound healing in damaged tissue, inhibiting the viral replication, repressing cancer and reducing drug resistance can be some of the important applications of the gene editing tools.

Intellectual Property & Genome Editing

Since the different gene editing tools possess the capacity to alter a wide range of genes, the usage of gene editing tools holds a lot of economic and commercial importance. Therefore, it becomes important to seek legal protection for the inventions associated with such systems and their applications. The patenting of new varieties of plants, transgenic plants and GMOs has been in existence for a very long time now and has seen significant commercial success. Now a new wave of genome editing mechanisms and their implications in different technological domains particularly in health care sciences is rapidly advancing. It still remains a matter of debate amongst various groups whether the inventions associated with molecular biology, particularly genetic engineering, are patentable under the existing patent laws or require specific amendments. Despite this fact, the inventions in the field of genome editing tools are accelerating at a significant rate. Similar is the case with the patent grant and licensing practices. For instance, the key intellectual property for ZFNs is with Sangamo while it is further licensed to Sigma-Aldrich and other companies for its commercial and therapeutic exploitation. Similarly, the TALENs have been licensed to Thermo-Fisher for their potential in the varied field of commercial value. The recent breakthrough of CRISPR/Cas9 in gene editing has triggered an ever-increasing interest in biotechnology-based innovation. While the battle for the legal rights is still on between UC Berkeley and the Broad Institute. The lucrative benefits that can be generated from the CRISPR/Cas9 system has not stopped the licensing/sub licensing of the CRISPR/Cas9 to different companies for their applications and further improvement. For instance, Excision Biotherapeutics is the first to exclusively license the new CRISPR systems discovered by Jennifer

Doudna's group (UC Berkeley) in 2016. Also, the European Patent Office has issued a "Notice of Intention to Grant '' to MilliporeSigma for CRISPR technology. At the same time French biotech company Cellectis has been granted a European patent to use CRISPR in T cells. Therefore, it is apparent that the recent emergence of the molecular biology related innovations are becoming a new charm for various biomedical solutions. Hence, the development of such new inventions and their legal protection is important in the everyday evolving biosciences.

Economics & Future Prospective

The economic benefits from the current genome editing technology appears to be far from the estimation. It is expected that the genome editing market will grow to $6.28 billion by 2022. The major growth is expected to be seen in the CRISPR technology while cell line editing and the research for diagnostic purposes is also estimated to contribute significantly. Further, the genetic manipulations directed therapeutics appear to be dominating soon that could bring about new treatment methods and a growth in the healthcare sector. For instance, the world's first attempt was made to edit the gene of the patient suffering from the Hunter's syndrome at UCSF Benioff Children's Hospital in Oakland, California.

Conclusion

So far, it is evident that the current gene editing mechanisms are more precise and possess a wide range of application without having greater ethical and physiological implications. Therefore, greater financial advantages and continuous improvements in this field are inevitable. Also, rational research and development, proper target identifications and intellectual property protection is significantly important for developing the next generation therapeutics, biological products, and processes.

References:

- Thomas G , Shannon J. S. et al ; Genome-Editing Technologies: Principles and Applications.
- Pellagatti A , Dolatshad H. et al ; Application of genome editing technologies to the study and treatment of hematological disease.
- Cloney R. Patent Law and Genome Engineering: A Short Guide to a Rapidly Changing Landscape.
- Jewell C. and Balakrishnan V .S. The battle to own the CRISPR–Cas9 gene-editing tool.
- The "Genome Editing/Genome Engineering Market – Global Forecast 2022" report, 2017.
- http://www.telegraph.co.uk/news/2017/11/16/first-gene-editing-attempted-human-body-cure-disease/

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