Genome Editing: A Review

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***Abstract*— The traditional healthcare system is at the doorstep for entering into the arena of molecular medicine. Genome editing is a recent method of making specific changes in the DNA. Editing Genomes with the Bacterial Immune System technology has emerged as a powerful technology for genome editing and is now widely used in basic biomedical research to explore gene function. This technology has been increasingly applied to the study and treatment of human diseases, by modifying human blood cells that are then put back into the body to treat several diseases as Cystic fibrosis, cancer and AIDS. The technology of genome editing involves cuts at specific DNA sequences with enzymes called engineering nucleases. Genome editing can be used to edit, remove, add or alter DNA in the genome. The enormous knowledge and ongoing research have now been able to demonstrate methodologies that can alter DNA coding. The techniques used to edit or change the genome evolved from the earlier attempts like nuclease technologies, homing endonucleases, and certain chemical methods. Molecular techniques like meganuclease, transcription activator-like effector nucleases (TALENs), and zinc-finger nucleases (ZFNs) initially emerged as genome-editing technologies. These initial technologies suffer from lower specificity due to their off-targets side effects. Moreover, from biotechnology's perspective, the main obstacle was to develop simple but effective delivery methods for host cell entry. Later, small RNAs, including microRNA (miRNA) and small interfering RNA (siRNA), have been widely adopted in the research laboratories to replace lab animals and cell lines. The latest discovery of CRISPR/Cas9 technology seems more encouraging by providing better efficiency, feasibility, and multi-role clinical application. This later biotechnology seem to take genome-engineering techniques to the next level of molecular engineering. This review generally discusses the various gene-editing technologies in terms of the mechanisms of action, advantages, and side effects.**

***Index Terms*— transcription activator-like effector nucleases**

INTRODUCTION

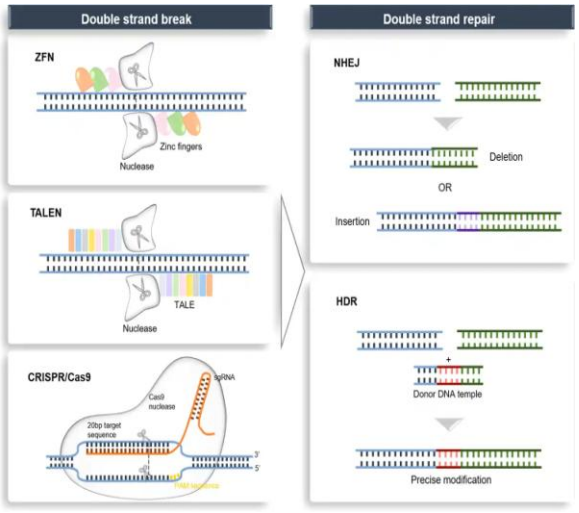
Over the last half century after post-DNA helical structure discovery, the world has seen a continuous staircase outburst of various molecular technologies, which are now heading forward toward translation into clinical and laboratory practice.1 Given the availability of sequencing platforms, acquired wisdom about the micro-mechanics at work within the genetic apparatus, and the introduction of user-friendly nanotechnologies, it was possible for next-generation scientists to manipulate the genetic codes at various levels.2 Over the last two decades we saw a plethora of molecular techniques, which allowed us to edit genes or their alter pathways, allowing humans for the first time to micro-edit the DNA codes and further to alter the mRNA fate through post-transcriptional modifications.3

Principally, genome-wide editing techniques can be interpreted as methods where DNA sequences are changed by deletions, mRNA processing, and post-transcriptional modifications to result in altered gene expression, leading to functional behavior of proteins.4, 5 Common to these methods are three basic steps, including mechanisms for genetic tool entry into the cell and later nucleus; altering gene transcription and onward processing function; and, finally, the end-output in the shape of a suppressed, over expressed, or simply an altered protein product.6, 7 From a holistic point of view, the techniques involve an apparently simplistic concept involving multiple receptor-ligand interactions; varying cell entry modes like lipofection, sonification, and transfection; and further downstream pathway effects. Furthermore, these technologies are variable in terms of their specificity and sensitivity, off-target effects, finances, and technique expertise. The body's immune response to accept the foreign genetic elements within the cells can lead to the rejection of foreign tissues.

Moreover, molecular knowledge, in terms of methodology differences, defining targetable diseases, innovative nanotechnology tools for gene editing, and ethical aspects, also needs to be understood. The platforms for these technologies are improving every day, with a plethora of new data appearing due to technology miniaturization and automation and newer discoveries to improve the yield and specificity of an edited product. Alongside the developmental improvement in genome-wide engineering the regulatory work-up, standardization protocols need to be devised to reduce inter and intra-method imprecision, defining the indications and contraindications of every technique to help improve the concept of personalized medicine.

Over the last few years, the exuberant development of genome editing has revolutionized research on the human genome, which has enabled investigators to better understand the contribution of a single-gene product to a disease in an organism. In the 1970s, the development of genetic engineering (manipulation of DNA or RNA) established a novel frontier in genome editing.1 Based on engineered or bacterial nucleases, genome editing technologies have been developed at a rapid pace over the past 10 years and have begun to show extraordinary utility in various fields, ranging from basic research to applied biotechnology and biomedical research.2 Genome editing can be achieved in vitro or in vivo by delivering the editing machinery in situ, which powerfully adds, ablates and "corrects" genes as well as performs other highly targeted genomic modifications.3,4 Targeted DNA alterations begin from the generation of nuclease-induced double-stranded breaks (DSBs), which leads to the stimulation of highly efficient recombination mechanisms of cellular DNA in mammalian cells.5,6 Nuclease-induced DNA DSBs can be repaired by one of the two major mechanisms that occur in almost all cell types and organisms: homology-directed repair (HDR) and nonhomologous end-joining (NHEJ),7 resulting in targeted integration or gene disruptions, respectively (Fig. 1).

Genome editing platforms and mechanisms for DSB repair with endogenous DNA. Genome editing nucleases (ZFNs, TALENs and CRISPR/Cas9) induce DSBs at targeted sites. DSBs can be repaired by NHEJ or, in the presence of donor template, by HDR. Gene disruption by targeting the locus with NHEJ leads to the formation of indels. When two DSBs target both sides of a pathogenic amplification or insertion, a therapeutic deletion of the intervening sequences can be created, leading to NHEJ gene correction. In the presence of a donor-corrected HDR template, HDR gene correction or gene addition induces a DSB at the desired locus. DSB double-stranded break, ZFN zinc-finger nuclease, TALEN transcription activator-like effector nuclease, CRISPR/Cas9 clustered regularly interspaced short palindromic repeat associated 9 nuclease, NHEJ nonhomologous end-joining, HDR homology-directed repair.



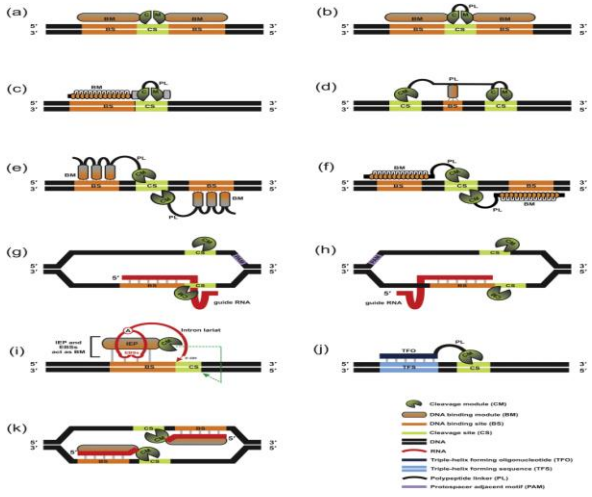
BRIEF HISTORY OF GENOME-EDITING EFFORTS

Genomes of eukaryotic organisms are composed of billions of DNA bases. The ability to change these DNA bases at precisely predetermined locations holds tremendous value not only for molecular biology, but also for medicine and biotechnology. Therefore, introducing desired changes into genomes, i.e., "genome editing", has been a long sought-after goal in molecular biology. To this end, the discovery of restriction enzymes that normally protect bacteria against phages in the late 1970s1,2,3 was a turning point that fueled the era of recombinant DNA technology. For the first time ever, scientists gained the ability to manipulate DNA in test tubes. Although such efforts drove a number of discoveries in molecular biology and genetics, the ability to precisely alter DNA in living eukaryotic cells came a few decades later.To this end, several key developments were revealed in the mid to late 1980s.

Initial targeted gene disruption studies in eukaryotic yeast cells4 followed with breakthrough work by Capecchi and Smithies in mammalian cells5,6,7. Their studies demonstrated that mammalian cells can incorporate an exogenous copy of DNA into their own genome through a process called homologous recombination5,6,7. Such targeted gene integration into the genome provided unprecedented power to characterize the functional roles of various genes in model organisms. However, the feasibility of this approach had several limitations. Firstly, the rate of spontaneous integration of an exogenous DNA copy was extremely low (1 in 103–109 cells)7. Secondly, the integration rate depended on cell types and cellular states. Finally, and most critically, the approach could result in random integration of the exogenous copy into undesired genomic loci ata frequency similar to or higher than that of the target site8.

GENOME EDITING REAGENTS

In general, genome editing tools using DSB nuclease-driven reactions (Fig. 1) can be divided into two groups. The first group consists of MNs, ZFNs and TALENs, which achieve sequence-specific DNA-binding via protein-DNA interactions [13], [42]. The second group is comprised of two sub-groups: (i) CRISPR/Cas9 and targetrons, which are RNA-guided systems [56], [57] and (ii) peptide nucleic acids (PNAs), triplex-forming oligonucleotides (TFOs), and structure-guided endonucleases (SGNs), which are DNA-based-guided systems [88], [89], [90], [91], [92]. A generalized comparison for the more commonly used genome engineering tools is presented in Table 1.



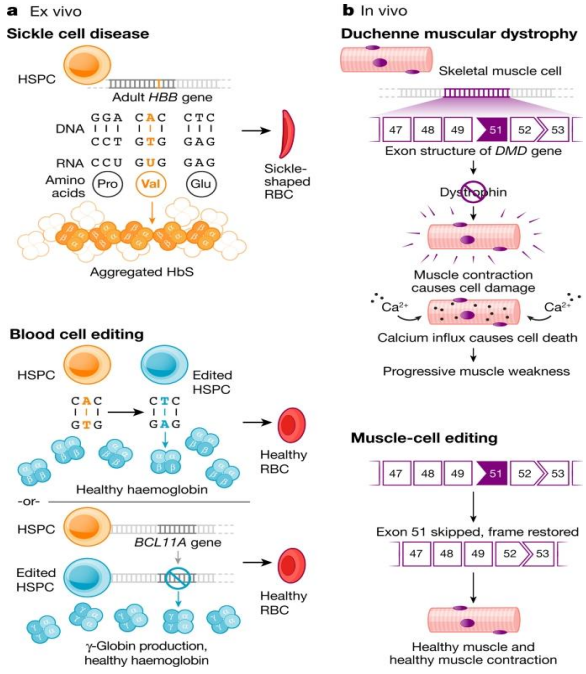
Genetic variation is a prerequisite of both natural and artificial selection. It allows populations to adapt to changing environmental conditions from generation to generation. Genetic variation occurs naturally through spontaneous mutations, processes during meiosis, and gamete combinations during fertilization or is induced by mutagenesis. These processes generate natural variation and are undirected but cannot be considered to be purely random.

A mutation is any change in genetic material that does not originate from the crossing of two individuals. Mutations can occur spontaneously or can be induced by external factors. DNA damage, which subsequently leads to the manifestation of mutations, is caused, for example, internally by mistakes during DNA replication, or can be induced by environmental factors, such as irradiation (e.g., UV light) or mutagenic substances. The occurrence of new mutations is not purely random since certain repair mechanisms, the local composition of the DNA sequence, and the chromatin state influence the retention of preceding DNA damage. DNA replication is a highly accurate biological process, but mistakes can occasionally occur when the DNA polymerase inserts a wrong base in the newly synthesized (daughter) strand. Most DNA polymerases directly correct mismatches through their proofreading function during DNA polymerization by sensing base mispairing. Nevertheless, some mismatches escape proofreading and are repaired after replication via DNA mismatch repair (MMR; Kunkel and Erie, 2015).

Genome editing, which involves the precise manipulation of cellular DNA sequences to alter cell fates and organism traits, has the potential to both improve our understanding of human genetics and cure genetic disease. Here I discuss the scientific, technical and ethical aspects of using CRISPR (clustered regularly interspaced short palindromic repeats) technology for therapeutic applications in humans, focusing on specific examples that highlight both opportunities and challenges. Genome editing is—or will soon be—in the clinic for several diseases, with more applications under development. The rapid pace of the field demands active efforts to ensure that this breakthrough technology is used responsibly to treat, cure and prevent genetic disease.

CHALLENGES FOR DELIVERY VECTORS

Fortunately, the potential to advance toward this goal has never seemed more achievable, even for the historically challenging field of neurologic disease. Indeed, the very first gene therapy approved in the United States was designed to treat inherited retinal dystrophy by transfer of wild-type RPE65 to the retinal pigmented epithelium of the eye (High and Roncarolo, 2019). Approved by the FDA in December 2017, Luxturna ushered in the long-anticipated era of gene therapy for diseases of the central nervous system (CNS). Adeno-associated virus (AAV), such as the AAV2 used in Luxturna, has become the vector of choice for delivery of many in-vivo gene therapy and gene editing applications, although other capsid proteins such as the serotype 9 (recombinant AAV2/9 or simply AAV9) are generally more efficacious for neuronal transduction in organs such as the brain (Ingusci et al., 2019). However, efficient delivery of genes and gene editing tools into neurologic tissues remains perhaps the most significant challenge for treatments of neurological diseases.



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