The gene is the most basic unit for genetic information transmission, and it determines all kinds of characteristics of life. With the completion of the human genome project and many biological genome sequencing projects, a large number of genomic information filled with mysteries of life are given to the scientists. The genome editing technology, a cutting-edge technology and different from the previous biological technologies, not only can be used to understand the gene function and interpret the genetic code. More importantly, it is also a subversive biotechnology that rewrites genetic code, leading to the deletion, insertion and replacement of nucleic acid sequences with high efficiency and specificity for a specific genome’s DNA (or RNA), thereby changing the characteristics of an organism.

The genome editing tool consists of two functional components. One, like a navigator, can quickly find the designated nucleic acid sequence within billions of bases, and the other, like a micro scalpel, can precisely manipulate...
nucleic acids. According to the differences in the principle of genome editing, there are three commonly used genome editing technologies including Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regulatory interspaced short palindromic repeat (CRISPR)/Cas-based RNA-guided DNA endonucleases (CRISPR/Cas) (1). Among the nucleases that possess genome editing capacity, CRISPR/Cas is the latest member with a high efficiency in genome editing and simplicity in cloning (2). As the efficiency and convenience of these three technologies are enhanced in turn, they are also known as the first, second and third generation of gene editing techniques (3). With the development of engineered nucleases such as ZFNs, TALENs and CRISPR, the field of human genome editing has evolved very rapidly. Targeted editing allows integrating an expression cassette into a safe genomic harbor, or correcting disease associated mutations. Gene correction, different with gene replacement, could restore the physiological expression and function of the gene, a long-sought goal of gene therapy (4).

Development of genome editing technologies

ZFN technology

The ZFN technology first appeared in the 90s, which was made up of a zinc finger protein fusion nuclease. Zinc finger protein is responsible for the recognition and binding of DNA. Once it binds to a specific nucleic acid sequence, nuclease, (FokI) it will precisely cut the nucleic acid sequence, which will produce a DNA double strand break (DSB) (3). The DSB is sealed by the error-prone non-homologous end-joining (NHEJ) pathway, which produces a small insertion or deletion mutations at the target point, causing a gene disruption. If the DSB is sealed by the high-fidelity homology directed repair (HDR) pathway, the targeted sequence can be edited precisely by integrating an exogenous DNA template flanked by homologous sequences to the nuclease target site (4). However, the accuracy of ZFN is based on the large zinc finger expression library from where the zinc finger protein is selected. It costs much time and labor, and is still difficult for use in large-scale application (3).

TALEN technology

The TALEN technology, developed in 2009, was found by the researchers that were looking into the amino acid sequence of the transcription activator like an effector encoded by the plant pathogen, Xanthomonas which composes of a number of similar modules. These have a constant correspondence to the nucleic acid sequences in the genome, as in each TALE module binds to one of the bases (5). By assembling these modules and fusing the FokI nuclease domain, the target DNA sequence can be targeted and precisely edited. Although the principle is similar to the ZFN technology, it is more flexible than the ZFN technology because it is easier to screen and build (6,7). The efficiency has been improved and the cost is lower. In 2012, Science magazine named it as one of the top ten major scientific and technological progresses.

CRISPR/Cas system

After years of in-depth study, the CRISPR/Cas system has been developed into becoming the most convenient and efficient genome editing tool as of 2012 (8). The CRISPR/Cas system plays an important role in the defense of foreign invasion of bacteria or archaea (8). The CRISPR/Cas system was reformed and contained two parts, including the single guide RNA (sgRNA) matching the target DNA fragment and the Cas9 nuclease (9,10). The CRISPR/Cas system has emerged as a potent new tool for targeted gene knockouts in bacteria, yeast, zebrafish and human cells (11). After the combination of sgRNA and the Cas9 protein, the Cas9 is brought to the target DNA sequence matching with sgRNA and the target DNA sequence is cut off by the Cas9 nuclease. The break of the DNA sequence is also repaired using NHEJ or HDR templates (12). NHEJ can introduce small deletions or insertions which leads to frame-shift mutations, while HDR allows for a larger and more precise perturbation (13). Unlike ZFN and TALEN, the CRISPR/Cas system is not artificial, and is a way of the natural adaptive immunity of bacteria. Targeted genome editing by Cas9 can efficiently produce knockout cells and organisms via NHEJ, but the efficiency of the precise sequence for replacement by the HDR is substantially lower (12). Chemical alterations to synthesized sgRNAs enhance genome editing efficiency in human primary T cells and CD34+ hematopoietic stem and progenitor cells (9). CRISPR/Cas not only can be used as a tool for gene therapy, but also a good helper for the exploration and discovery of various kinds of disease mechanisms. Its application covers many fields of life.
science, such as farming, forestry, animal husbandry, and so on. The emergence of the CRISPR technology has rapidly become the focus of life science research all over the world. Although the genome-wide specificities of CRISPR-Cas9 systems remain to be fully defined, the ability of precise positioning and efficient manipulation of the genomic sequences will greatly enhance biological and medical research and promote the treatment of human diseases (14). CRISPR-Cas9 system may be used to: (I) introduce stable changes into genomes, (II) activate or silence the expression of a gene of interest, and (III) visualize specific sites in genomes of living cells. Many tools are derived from CRISPR-Cas9 system have been successfully employed in generate disease models in animal and cell for research, e.g., specific types of cancer (15).

**Application of genome editing technologies**

As a new subversive biotechnology, genome editing is closely related to the biological economy. Its development and application are related to the national economy and the people's livelihood. It has great potential in biological medicine, bioagriculture, bioenergy, biosynthesis, biomaterials and biosafety.

**Genome editing technologies in genetic diseases**

The current medical technologies and means of usage are still difficult in the application of overcoming hereditary diseases. Gene therapies based on the genome editing technique can accurately repair genetic mutations into normal genes. As a number of studies reported life-long cure has been proved more safety than traditional gene therapy at least in animal models through the strategy (16-19). Genome editing technology has been proven to cure the fatal genetic diseases completely and can fundamentally overturn the current situation of patients being on life-long medications for hereditary diseases. Genome editing plays an important role in gene therapy, cell therapy, and the screening and development of new drugs.

Site-specific genome editing has great therapeutic potential for achieving long-term, stable gene expression. Duchenne muscular dystrophy (DMD) is caused by mutations in the Dmd coding dystrophin gene. The mdx mouse harbors a premature termination codon in exon 23 of the DMD locus and serves as a useful model for DMD (16). Delivery by recombinant adeno-associated virus (AAV) of CRISPR-Cas9 coupled with sgRNAs flanking the mutated Dmd exon23 resulted in an excision of the intervening DNA and restoring the Dmd reading frame and the dystrophin expression in the terminally differentiated skeletal muscle fibers and cardiomyocytes, as well as muscle satellite cells, in neonatal and adult mice after a local or systemic delivery. This study shows that in vivo genome editing can repair damage caused by mutant genes for therapeutic in dystrophic mouse models (17). Transient expression of Cas9, sgRNA and a co-injected ssDNA by non-viral hydrodynamic injection resulted in initial expressions of the wild-type Fah protein in ~1/250 liver cells. Expansion of Fah-positive hepatocytes rescued the body weight loss phenotype a mouse model of hereditary tyrosinemia type I (HTI). These data points indicated that the CRISPR-Cas9 system can be used in adult animals and has potential for correction of human genetic diseases (18).

Administration of AAV vectors that encode a donor construct and a ZFN pair targeting (process) the albumin locus was successfully corrected the phenotype of the mouse models with hemophilia types A and B. Thereby, we achieved the long-term expression of human factors VIII and IX (hFVIII and hFIX) in mouse models with hemophilia types A and B at the therapeutic level (19). By using the same targeting reagents in wild-type mice, cause Fabry and Gaucher diseases and Hurler and Hunter syndromes by lysosomal enzymes deficiency (19). Genovese and his colleagues demonstrated the therapeutic potential of their strategy by targeting a corrective cDNA into the IL2RG gene of hematopoietic stem cells (HSCs) from healthy donors and a subject with X-linked Severe Combined Immunodeficiency (SCID-X1). Gene edited HSCs not only has normal hematopoietic function, but also produces functional lymphocytes with more growth advantages (4).

In addition to the preclinical studies, many European and American countries have carried out clinical trials of gene therapy based on genome editing. In November of 2017, Sangamo Therapeutics announced the first in vivo genome editing clinical trial for Hunter’s syndrome had been completed (20).

**Genome editing technologies in cancers**

Cancer is a multistep process that involves mutations and other alterations in oncogenes and tumor suppressor genes (21). Reversing the alteration of oncogenes or tumor suppressor genes may effectively inhibit the occurrence and development of tumors. CRISPR-Cas9 is a versatile
genome editing technology for manipulating the genomes with unprecedented precision (22, 23).

In recent years, tumor research has seen a breakthrough in immunotherapies which are represented by the chimeric antigen receptor T (CAR-T) using cell technology. Modified autologous T cells with chimeric antigen receptors (CAR) transfused in patients can clear tumor cells in vivo and have a very high cure rate for some tumors (24-26). Although CAR-T technology has achieved an initial success, there are still some unsolved problems and potential risks. (I) The poor function of autologous T cells in the older cancer patients does not meet the requirements of CAR-T therapy. (II) The effects are very poor for solid tumors other than hematological malignancies. (III) The method used in the preparation of CAR-T cells may cause the transfused T cells to occur T cell lymphoma (27, 28). Using gene-disrupted allogeneic T cells as universal effector cells provides this alternative. Ren et al. generated gene-disrupted allogeneic CAR-T cells deficient of TCR, HLA class I molecule and PD1 by combining lentiviral delivery of CAR and electro-transfer of Cas9 mRNA and gRNAs targeting endogenous TCR, β-2 microglobulin and PD1 simultaneously, which could provide an alternative as a universal donor to autologous T cells and potentially improves current CAR-T cell therapy against cancers (29). The function of human CAR-T cells can be affected by PD-L1 expression in the tumor cells, which weaken the fight for tumors. To overcome this suppressed anti-tumor response, Rupp et al. developed a protocol for a combined Cas9 RNP-mediated gene editing and lentiviral transduction process to generate PD-1 deficient anti-CD19 CAR T cells, which augmented CAR-T cell mediated tumor cell killing capabilities in vitro and enhanced clearance of PD-L1+ tumor xenografts in vivo (30). Poiret et al. demonstrated that TALEN-mediated genome editing could be used in output third-party therapeutic CAR T-cells in a large-scale test. These engineered T cells can be premanufactured from suitable third-party donors, modification T cell via genetic engineering with retrovirus-encoded CAR, which will render the ability of killing tumor cell. Furthermore, the engraftment of these engineered T cells into any patient can be further supported when administered with a chemotherapeutic agent to whom they have been engineered for resistance (31). Zhang et al. successfully generated lymphocyte activation gene-3 (LAG-3, negative regulator of T cell activity) knockout CAR-T cells using the CRISPR-Cas9 system, which displayed the robust antigen-specific antitumor activity in the cell culture, and in the murine xenograft model (32). Theoretically, genome editing technology can also solve the poor efficacy of the existing CAR-T technology for solid tumors and the risk of T cells forming tumors themselves. There is no doubt that genome editing technology combined with CAR-T strategy will become the first choice for cancer gene therapy in the future.

**Genome editing technologies in virus infections**

Genome editing technologies have also played a magical role in fighting persistent viral infections. Although the treatment of acquired immunodeficiency syndrome (AIDS) caused by human immunodeficiency virus (HIV) infection has made great progress, there is no radical cure except bone marrow transplantation. Hu et al. demonstrated that specific targets within the HIV-1 LTR U3 region were efficiently edited by Cas9/gRNA, which could inactivate viral gene expression and replication in latently infected microglial, promonocytic, and T cells (33). CXCR4 is a co-receptor for the HIV-1 infection and mediates viral entry into human CD4+ cells by binding to envelope protein, gp120. Hou et al. demonstrated that human CXCR4 genes are efficiently disrupted by CRISPR-Cas9 system, which led to HIV-1 resistance of human primary CD4+ T cells (34). CCR5 is the major coreceptor for human immunodeficiency virus (HIV). Tebas et al. suggested that the infusion of autologous CD4+ T cells in which the CCR5 gene was rendered permanently dysfunctional by ZFN was safe (35).

Hepatitis B virus (HBV) infection remains a major global health problem because current therapies rarely eliminate HBV infections to achieve a complete cure. Peng et al. reported that CRISPR/Cas9 was designed to target HBV specific DNA sequences to suppress HBV replication and to cause HBV genome mutation (36). Lin et al. successfully reduced the production of HBV core and surface proteins in Huh-7 cells transfected with an HBV-expression vector created by CRISPR/Cas9 system with the HBV-specific gRNAs (37). Dong et al. suggested that synthesized sgRNAs targeting the conserved regions of HBV could reduce the viral production in Huh7 cells as well as in HBV-replication cell HepG2.2.15. They further demonstrated that the designed CRISPR/Cas9 system can accurately and efficiently target HBV cccDNA and inhibit HBV replication (38). In summary, CRISPR/Cas9 system is likely to become a routine therapeutic strategy against viral infection in the future.
**Genome editing technologies in bio-agriculture**

Genome editing tools enable efficient and accurate genome manipulation. Not only for medical research, but also used in agriculture, including improved disease resistance, productivity or breeding capability of livestock and crops (39). To meet current challenges in agriculture, genome editing using sequence-specific nucleases (SSNs) as a powerful tool for basic and applied plant biology research (40). China is a big agricultural production country and used as large amount of seeds. At the present, the limitations of traditional breeding technology are difficult to carry the burden of the enormous challenges of future food security. Biological seed industry is the strategic and basic core industry of the country and also a fundamental guarantee for national food security and sustainable development of agriculture. Molecular breeding technology based on genome editing is actually not a genetically modified technology, but it is only artificially concentrated and accelerated the process of spontaneous mutation and artificial mutation screening. The new varieties obtained are genetically not fundamentally different from natural mutations and cannot be detected by existing means. Cadherins are receptors of Bacillus thuringiensis (Bt) Cry1A toxins in several lepidopteran insects. Disruption of the cadherin gene in H. armigera has been genetically linked to the resistance of the Bt toxin Cry1Ac. The cadherin gene from the Cry1Ac-susceptible SCD strain of H. armigera was successfully knocked out by using the CRISPR/Cas9 genome editing system (41). Kishi-Kaboshi et al. constructed transgenic chrysanthemum plants expressing the yellowish-green fluorescent protein gene from Chiridius poppei (CpYGFP), selected two sgRNAs to target different positions in the CpYGFP gene, obtained transgenic calli containing mutated CpYGFP genes (CRISPR-CpYGFP-chrysanthemum) (42). Odipio et al. generated multi-allelic mutations in cassava by CRISPR/Cas9 system, which was highly efficient and simple. Modification of Phytoene desaturase (MePDS) generated visually detectable mutated events in a short time frame of 6–8 weeks (43). Europe and the United States have obtained 6 CRISPR edited foods (including corn, soybeans, mushrooms, rapeseed and potatoes) which have been approved.

**Genome editing technologies in microorganism**

Microorganism genome editing is the foundation and source of CRISPR/Cas9 editing technology system, which is of great significance in the development, innovation and application of editing technology.

Model microbial *Escherichia coli* are the most commonly used research and screening platforms for gene editing technology. All the editing techniques have been proved successful in Escherichia coli tests which were then transplanted into plants and mammalian cells. Xu et al. investigated the use of the *Streptococcus pyogenes* CRISPR-Cas9 system in editing the genome of *Clostridium cellulolyticum*, a model microorganism for bioenergy research. Double-strand breaks induced by wild-type Cas9 system were over the threshold of NHEJ, leading death of *Clostridium cellulolyticum* (44). Wolf et al. adopted the CRISPR-Cas9 system for *Actinoplanes* sp. SE50/110, the producer of the diabetes type II drug acarbose. The CRISPR-Cas9 system was successfully shown by the scarless and antibiotic marker-free deletion of the tyrosinase MelC, which causes *Actinoplanes* sp. SE50/110 no longer produce this pigment and therefore the supernatant does not darken (45). Solventogenic clostridia are important industrial microorganisms that produce various chemicals and fuels. Li et al. demonstrated that *Streptococcus pyogenes* dCas9-mediated gene regulation control system, pDCASclos, was used in a CRISPRi strategy to successfully inhibit the expression of spo0A in *Clostridium acetobutylicum* and *Clostridium beijerinckii*, which will greatly accelerate future progress in the understanding and manipulation of metabolism in solventogenic clostridia (46). Mougiakos et al. apply *Streptococcus pyogenes* Cas9 (spCas9)-based genome editing to a moderate thermophile, i.e., *Bacillus smithii*. The spCas9 is inactive *in vivo* above 42 °C. Subsequent transfer to 37 °C allows for counter selection through production of active spCas9, which introduces lethal double-stranded DNA breaks to the nonedited cells (47).

**Future directions**

Through genome editing (ZFNs, TALENs and RNA-guided DNA endonucleases), the biosynthesis and metabolic pathways of microorganisms can be transformed to obtain inexhaustible drug molecules and new structures to become an important source of innovative drugs in the future. These genome editing tools are wildly employed in the investigation of gene function, genetic mechanisms of diseases, discovery of new therapeutic targets, and the development of disease models. Moreover, these genome editing technologies have great potential in gene therapy. Gene therapy has historically been defined as the addition
of new genes to human cells. However, the emergence of genome-editing technologies allows for a precise manipulation of human genome sequences for therapeutic effects. Indeed, these emerging technologies have dramatically expanded the ability to manipulate and study model organisms and support the promise of correcting the genetic causes behind many diseases including diabetes, metabolic syndrome, cardiovascular and cerebrovascular diseases, Alzheimer’s disease, macular degeneration of retinitis pigmentosa, and senile diseases.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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