Genome-editing technologies: Advancement, clinical applications, and ethical concerns

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Abstract

Genome editing is a powerful technology capable of precisely manipulating somatic and germline genomic sequences. The field is progressing at a rapid pace with unprecedented applications in biology and medicine. This systematic review represents a guide to different mechanisms, tools, and delivery systems used in genome editing. In addition, related ethical concerns are highlighted. So far, the most recent developed tool, clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeats-associated enzyme, represents the most widely used approach owing to its simple application and enhanced efficiency. Hence, the major part of the review focuses on the clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeats-associated enzyme technology and its diverse applicability.

Keywords:

clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeats-associated enzyme, delivery systems, genome editing, germline, somatic

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Introduction

Genome editing has emerged as an invaluable approach of genetic engineering in which a specific DNA sequence is modified using custom-designed programmable nucleases that produce site-specific DNA double-strand breaks (DSBs). DSBs can be repaired by endogenous DNA repair systems [homology-directed repair (HDR) or nonhomologous end joining (NHEJ)], often causing site-specific genome modifications to allow a genetic material to be inserted, removed, or altered at a particular location in the genome (Sakuma and Woltjen, 2014). Genome editing modifies the genome at a precise, predetermined locus; therefore, it avoids random integration, the major problem of classical gene therapy, which may lead to malignant transformation (when transgene integrated near proto-oncogenes converting them into oncogenes) or serious gene dysfunction (when transgene integrated into specific gene disturbing its activity) (Porteus *et al.*, 2006).

Owing to their wide applicability, genome-editing technology invades all aspects of molecular biology and genetic engineering. Applications of genome editing are so versatile to the point that it is impossible to be thoroughly covered in one article.

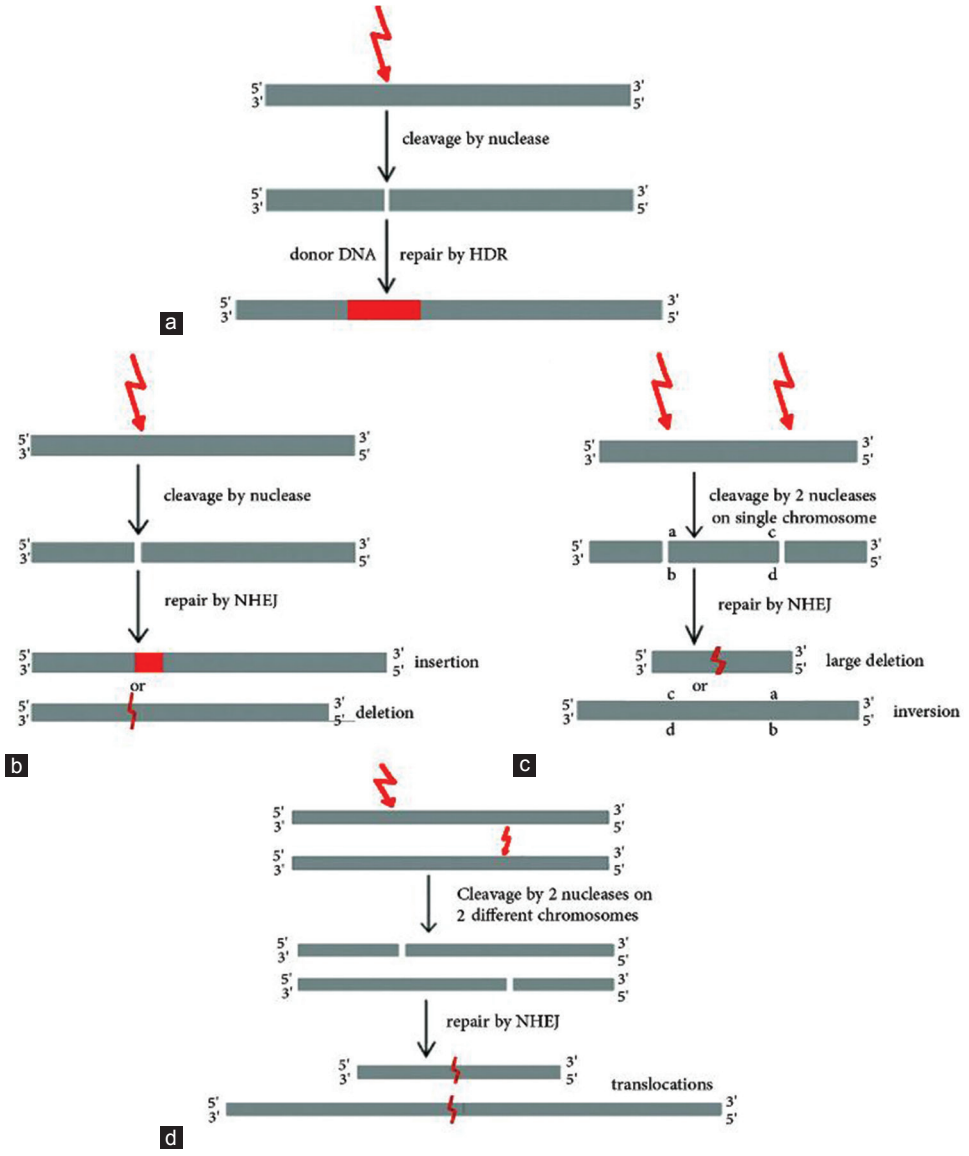
To prepare this systematic review, we have used PubMed and Google Scholar advanced engines to search for English language articles of human genome editing published from 2015 till present. The search resulted in more than 2000 different items denoting for the unprecedented pace of the genome-editing technology.

Cellular repair machinery of double-strand breaks

The cell can repair DSBs by either HDR or NHEJ based on the cell state and the existence of a repair template. Editing DSB by HDR requires presence of a donor template. HDR naturally uses the sister chromatid as a template for DSB repair. The template is rarely the undamaged homologous chromosome (resulting in loss of heterozygosity) (Jeggo, 1998). However, HDR machinery can also utilize exogenously provided single-stranded or double-stranded DNA molecules flanked by homology arms (sequences identical to those flanking the break site) (Fig. 1a). Hence, any sequence variations in the donor template will be incorporated into the cut region, which can be applied to correct disease-causing mutations. Single-stranded oligonucleotides can be easily synthesized with a small length as little as 80 bp. Moreover, they are associated with induced rate of HDR (Chen *et al.*, 2011).

On the contrary, NHEJ repairs DSBs in the absence of template through direct religation of the cleaved ends. Although NHEJ has high fidelity (>70%), the nuclease is still active after rejoining and recuts the site again

Figure 1



Nuclease-based genome editing. When a nuclease creates a DNA double-stranded break (DSB), the cellular repair machinery acts to fix it. (a) Repair of a DSB by homology-directed repair (HDR) using a provided donor DNA (red-colored) incorporates the sequence variations specified by the donor template. (b) Repair of a DSB by nonhomologous end joining (NHEJ) leads to insertions/deletions at the break site. (c) When two DSBs on one chromosome are edited by NHEJ, chromosomal deletions or inversions can be created. (d) When two DSBs occur at two different chromosomes, translocations can be developed.

creating another DSB. Ultimately, repeated repair of the same DSB by NHEJ leads to formation of small insertions or deletions (indels) at the cut region. The size of indels is usually 1–15 bp, but it can be much larger (Fig. 1b). Indels often disrupt the open reading frame resulting in gene knockout. It might be used for exploring the function of genes or other genetic elements (Hendel *et al.*, 2014).

If two DSBs are originated simultaneously on one chromosome, NHEJ will result in the deletion of the sequence intervening the two DSBs. This approach can be used to remove large DNA sequences for therapeutic applications (Ousterout *et al.*, 2015). Inversion of the sequence in between the two DSBs may also take place if the deletion frequency is ~1% (Fig. 1c). If two DSBs are simultaneously created on two different chromosomes, gross chromosomal translocations can occur (Fig. 1d) (Lee *et al.*, 2010).

Generally, NHEJ is more frequent than HDR. NHEJ is thought to be active during the different phases of cell cycle observed in dividing and post-mitotic cells. In contrast, HDR is largely restricted to actively dividing cells (Branzei and Foiani, 2008). Cell cycle regulation can be bypassed for slowly dividing cells via enhancing their mitotic division using pharmacologic agents *ex vivo*. However, post-mitotic cells are unlikely amenable to such manipulation (Iyama and Wilson, 2013).

Programmable nucleases

To date, four major classes of programmable nucleases, meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/clustered regularly interspaced short palindromic repeats-associated enzyme (Cas) systems (CRISPR/Cas), have been developed to create site-specific DSBs (Table 1) (Adli, 2018).

Meganucleases

Meganucleases (homing endonucleases) are endodeoxyribonucleases found in all kingdoms of life. They are mainly characterized by their large recognition site (14–40-bp) representing the most specific naturally occurring restriction enzymes (Stoddard, 2011). The first identified meganuclease is I-SceI. This nuclease is located within a genetic marker called 'omega', from which the term 'meganucleases' is derived. Moreover, the expression 'meganucleases' refers also to the large recognition site of such nucleases. Omega corresponds to an intervening sequence within the mitochondrial gene which encodes the large ribosomal RNA subunit. Interestingly, when I-SceI is expressed, it cleaves its target site that is located in the homologous allele of the host gene that lacks the Omega marker. Hence, the term 'homing' is generated. The resulting DSB may be repaired via HDR using the omega-plus allele as a template. Thus, the originally intron lacking allele (I^-) is converted to an allele containing the intron (I^+) (Fig. 2) (Jacquier and Dujon, 1985).

I-SceI was first utilized to introduce DSBs in 1994 (Rouet *et al.*, 1994). However, it is hard to find a meganuclease targeting the desired sequence. Two main approaches have been developed to create meganucleases with new specificities: (a) introducing small variations to the amino acid sequence of the existing meganucleases and (b) fusing domains from different enzymes. These two approaches can also be combined to create new enzymes. Despite such efforts, very limited genomic sequences could be targeted using meganucleases. Moreover, engineering novel meganucleases is a challenging process, limiting the use of this platform (Silva *et al.*, 2011).

Zinc finger nucleases

ZFNs are a class of engineered DNA-binding proteins that enable targeted editing of the genome by creating DSBs in DNA at user-specific locations. ZFNs are composed of the nonspecific cleavage domain of the FokI restriction endonuclease and a tandem array of the DNA-binding domain of zinc finger proteins (ZFPs). ZFNs contain 3–6 zinc fingers, where each finger interacts with three DNA bases. Importantly, ZFs can recognize all known nucleotide triplets. FokI must dimerize to cleave DNA, and thus a pair of ZFNs is required to form an active nuclease; each monomer binds one of the adjacent half-sites separated by spacer of 5–7-bp. This requirement for dimerization doubles the length of the recognition sites, which substantially increases the specificity of ZFNs. Ultimately, ZFNs bind DNA sequences of 9–18-bp and the paired ZFNs achieve a specificity of 18–36-bp at the target site (Fig. 3) (Urnov *et al.*, 2010).

Since their engineering in 1991 (Pavletich and Pabo, 1991), ZFNs represented the predominant tool of genome editing for over 10 years until TALENs were emerged in 2011 (Bogdanove and Voytas, 2011).

Transcription activator-like effector nucleases

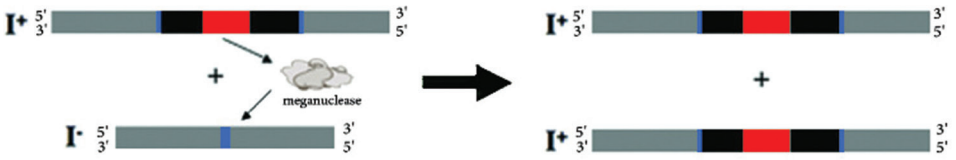
Like ZFNs, TALENs harbor the FokI nuclease domain; however, they contain a customized tandem array of another class of DNA-binding domains called transcription activator-like effectors (TALEs), which are derived from the phytopathogenic bacteria Xanthomonas spp. Each TALE includes a central domain consisting of 33–39 amino acids and recognizes a single base pair. The nucleotide specificity

Table 1 Comparison of programmable nucleases

	Meganucleases	ZFNs	TALENs	SP CRISPR/Cas9
Created breaks	With 3′ overhangs	With 5′ overhangs	With 5′ overhangs	Blunt
Cytotoxicity	Not cytotoxic	May be cytotoxic	Not cytotoxic	Not cytotoxic
Mechanism of recognition	Protein–DNA	Protein–DNA	Protein–DNA	RNA–DNA
Length of target site (bp)	14-40	18–36	32–48	23
Restriction in target site	Very limited sequences could be targeted	G-rich (due to natural preference of zinc fingers)	Start with T and end with A	End with NGG or NAG
Size	1 kb	~1 kb×2	~3 kb×2	4.2 kb Cas9+0.1 kb gRNA
Ease of design	Extremely difficult	Difficult	Easy	Easy
Specificity	Small number of mismatches tolerated	Small number of mismatches tolerated	Small number of mismatches tolerated	Multiple mismatches tolerated

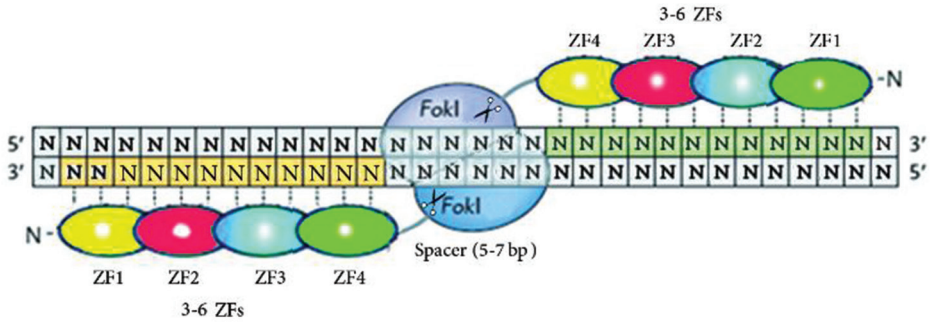
CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeats-associated enzyme 9; SP, *Streptococcus pyogene*; TALENs, transcription activator-like effector nucleases; ZFNs, zinc finger nucleases.

Figure 2



Action of meganucleases. An intron (black-colored) containing a meganuclease gene (red-colored) resides within a host gene (grey-colored). When the homing endonuclease is expressed, it binds to its target site (blue-colored) in the intron lacking allele (I^-) generating a double-strand break (DSB). The DSB may be restored via HDR using the intron containing allele (I^+) as a template. This in turn leads to generation of the intervening sequence containing the meganuclease gene in the restored allele.

Figure 3



A schematic presentation of a zinc finger nuclease (ZFN) pair. Each ZFN is composed of the DNA-binding domain of a zinc finger protein (ZFP) at the amino terminus and the FokI nuclease domain at the carboxyl terminus. ZFN pairs typically contain 6–12 zinc fingers (ZFs). Each ZF binds three DNA bases. Ultimately, ZFNs pairs bind DNA sequences of 18–36-bp. N represents any nucleotide.

of each repeat domain is determined by the two amino acids at positions 12 and 13, known as repeat variable diresidues (RVDs). TALENs can be engineered to target virtually any sequence with 5' T, specified by the constant N-terminal domain, for each array. Four different repeat variable diresidues modules – namely, Asn–Ile, His–Asp, Asn–Asn, and Asn–Gly – are most widely used to recognize adenine, cytosine, guanine and cytosine and thymine, respectively. As for ZFNs, a pair of TALENs is needed to recognize a single target site. Each TALEN usually defines DNA sequences of 16–24-bp (32–48-bp when TALEN pairs dimerize) (Fig. 4) (Bogdanove and Voytas, 2011).

Importantly, FokI nickases have been successfully developed with ZFs and TALEs inducing the nuclease specificity, while reducing the related toxicity. Nickases are created by inactivating the catalytic activity of one FokI monomer in the nuclease dimer. Paired nickases produce two single-strand breaks on different DNA strands, generating a composite DSB and thus doubling the nuclease specificity (Kim *et al.*, 2012).

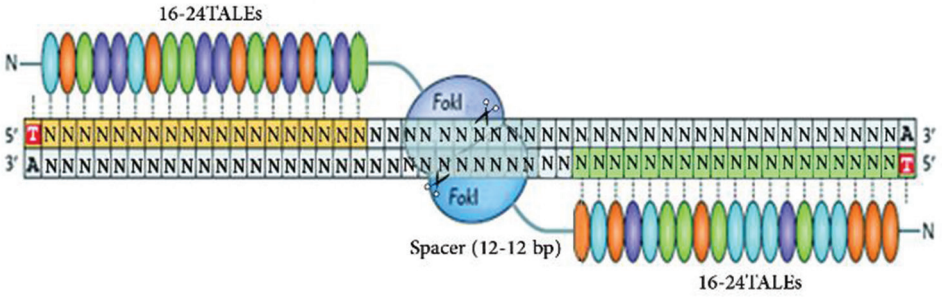
Clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeats-associated enzyme

In bacteria and archaea, CRISPR/Cas systems provide adaptive immunity against invading phages or plasmids. They are mainly divided into class 1 and class 2, where each is further subdivided into several types and subtypes. Different types of class 2 systems have successfully been utilized in genome editing. Class 2 systems currently consist of types II, V, and VI (Hsu *et al.*, 2014). CRISPR/clustered regularly interspaced short palindromic repeats-associated protein 9 (Cas9), derived from *Streptococcus pyogenes* and other bacteria, was the first class 2 system to be discovered, characterized, and used for genome editing (Jinek *et al.*, 2012).

In type II CRISPR systems, CRISPR regions consists of a targeting sequence (crRNA sequence) and a Cas9 nuclease-recruiting sequence representing together the small guide RNA (sgRNA). crRNA contains the 20-bp sequence that binds the target site known as spacer. sgRNA is complexed with Cas9 to form an active DNA endonuclease. RNA loading induces conformational rearrangements in Cas9 forming a central channel that may accommodate target DNA. Cas9 recognizes a 3-bp protospacer adjacent motif (PAM). The resulting endonuclease cleaves a 23-bp target DNA sequence that is complementary to 20-bp sequence in the sgRNA (spacer) and 5'-NGG-3' or, to a lesser extent, 5'-NAG-3' PAM sequence (Fig. 5) (Jinek *et al.*, 2012).

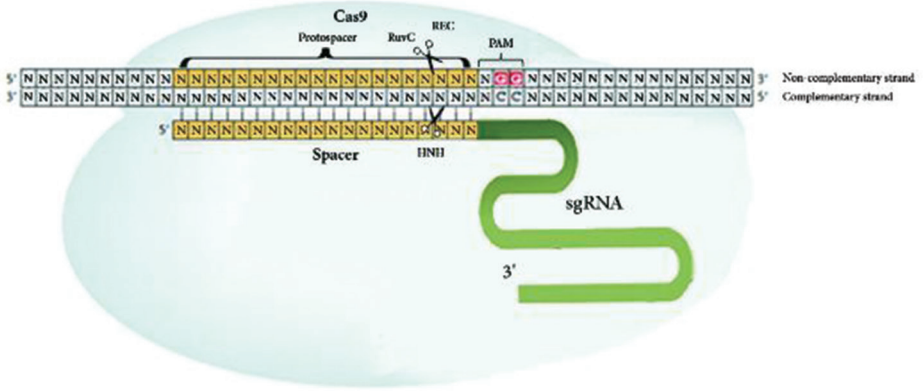
Cas9 consists of two major lobes, recognition lobe and nuclease lobe. The nuclease lobe itself consists of two catalytic domains, RuvC and HNH, where each nicks one strand of the target DNA sequence. The HNH

Figure 4



A schematic presentation of a transcription activator-like effector nuclease (TALEN) pair. Each TALEN is composed of a tandem array of transcription activator-like effectors (TALEs) at the amino terminus and the FokI nuclease domain at the carboxyl terminus. Target sequence must have T residue at the 5' end, recognized by the constant amino terminus domain of each TALEN. TALEN pair usually defines DNA sequences of 32–48-bp. N represents any nucleotide.

Figure 5



A schematic presentation of clustered regularly interspaced short palindromic repeats (CRISPR)/clustered regularly interspaced short palindromic repeat-associated enzyme 9 (Cas9) system (CRISPR/Cas9). CRISPR/Cas systems contain two main components: a small guide RNA (sgRNA) and a CRISPR-associated endonuclease (Cas protein). The sgRNA contains a user-defined 20-bp spacer that binds the target sequence to be modified. Cas9 consists of two lobes, recognition (REC) lobe and nuclease (NUC) lobe. The nuclease lobe contains two catalytic domains, HNH and RuvC cutting the sgRNA complementary strand and the displaced strand, respectively.

domain cuts the DNA strand complementary to the sgRNA and the RuvC domain cleaves the displaced strand, yielding a site-specific DSB. Importantly, inactivation of one of these domains results in a partially inactivated Cas9 or Cas9 nickases that can generate DNA single-strand breaks, whereas inactivation of both domains inhibits the Cas9's catalytic activity, resulting in dead clustered regularly interspaced short palindromic repeats-associated protein 9 (dCas9) (Qi *et al.*, 2013).

Three orthologs of Cas9 with longer PAM sequences, derived from *Streptococcus thermophilus* (PAM: NNAGAAW) (Cong *et al.*, 2013), *Neisseria meningitides* (PAM: NNNNGATT), and *Staphylococcus aureus* (PAM: NNGRR) (Ran *et al.*, 2015). Accordingly, they are associated with reduced off-target activity and increase the possibility to find a PAM sequence in the desired gene.

Type V includes Cas12a (Cpf1) and Cas12b (C2c1). Interestingly, they contain only a single nuclease domain (RuvC) that cleaves both DNA strands (Begemann *et al.*, 2017). On the contrary, type VI contains Cas13a and Cas13b (C2c2). Importantly, Cas13 nucleases target RNA rather than DNA providing new areas for genome-editing applications (Yan *et al.*, 2018).

Unlike other programmable nucleases, CRISPR/Cas does not require protein engineering, and it is apparently simple to design RNA-guided molecules. Coupled with its efficiency, CRISPR/Cas has been quickly adopted, revolutionizing the field of genome editing. Interestingly, since its emergence in late 2012, more than 9000 related research articles have been published. Additionally, 2018 is the year in which CRISPR-Cas clinical trials have been started (Adli, 2018).

A main issue is that CRISPR/Cas could tolerate certain nucleotide mismatches, increasing its off-target activity. To this end, forward steps have been taken to

increase its targeting specificity. Notably, using a pair of Cas9 nickases doubles the target site length, reducing the off-target activity, while inducing the on-target activity. Moreover, Cas9 nickases increase the ratio of HDR to NHEJ. However, target sequences have to contain two PAM sequences, limiting the availability of targetable sites (Mali *et al.*, 2013). In addition, both sgRNA truncation and elongation have been shown to reduce the off-target activity and result in better on-target to off-target ratios. Addition of two target independent guanine nucleotides at the 5' terminus yields much more specific sgRNAs at least in human cells. Moreover, truncated sgRNAs with 17-nt (rather than 20-nt) have higher specificity (Koo *et al.*, 2015).

Hybrid nuclease platforms

Two forms of hybrid nucleases have been furtherly developed in an attempt for inducing the activity and improving the specificity of nucleases.

Clustered regularly interspaced short palindromic repeats-associated protein 9-FokI nucleases

In this platform, the two nuclease domains in a Cas9 pair are catalytically inactivated creating dCas9s. Then they fuse to the nuclease domains of FokI, forming a dCas9-FokI pair. In this case, DNA targeting is achieved by two gRNAs and dCas9s, and DNA cleavage is executed by the dimerized FokI domains. Although off-target activity is significantly diminished compared with Cas9 nickases, reduced on-target activity is also observed (Guilinger *et al.*, 2014).

Mega-transcription activator-like nucleases

Mega-TALs are constructed by fusing minimal TALE DNA-binding domains to meganucleases. This potentiates the meganuclease activity toward targets for which it has low affinity. The activity of mega-TALs is reliant not only on the meganuclease but also on the TALE array, increasing the on-target activity and reducing the off-target cleavage. Mega-TALs are expressed as a compact single chain of ~2 kb facilitating their delivery using all current viral and nonviral vectors. However, using of this platform is restricted to have a meganuclease with considerable affinity for target site (Boissel *et al.*, 2014).

Base editing and other applications of catalytically inactive clustered regularly interspaced short palindromic repeats-associated protein 9 (dead clustered regularly interspaced short palindromic repeats-associated protein 9)

Base editing is a recently developed approach of genome editing that enables programmable substitution of one DNA base, without requiring DNA cleavage or donor template. The first base editor system was developed by Komor *et al.* (2016). Base editors are chimeric proteins composed of a DNA targeting molecule and a catalytic domain capable of deaminating a cytidine and adenosine into uridine and inosine that are finally replaced by thymine and guanosine, respectively. In most such systems, the DNA targeting module is based on dCas9 guided by a sgRNA molecule. Cas9 nickase can also be used as the targeting module, resulting in high frequencies of base editing. This may be accomplished via using the edited strand as a template to repair the nicked strand or via providing better accessibility for the deaminase. However, this increased catalytic activity resulted in an increased indel frequency. Importantly, the desired conversion occurs within a five-nucleotide activity window at the nontarget strand of the selected genetic locus in case of Cas9 deaminases (Eid *et al.*, 2018). Intriguingly, a base editor system has been recently repurposed to edit adenosine to inosine in mRNA sequences via the use of a catalytically inactive Cas13 protein and a deaminase enzyme (Cox *et al.*, 2017).

However, it skips this review scope. It is noteworthy that the applications of dCas9 extend beyond genome editing by virtue of its guidable capacity. dCas9 can recruit diverse effector proteins to specific DNA sequences mediating several kinds of manipulation. These guidable proteins mainly include transcriptional activators and repressors (to regulate gene expression), epigenetic modulators (to alter epigenetic information), fluorescently labeled proteins (for chromatin imaging), and chromatin structure rewiring proteins (to modify the 3D chromatin structure in the nuclear space) (Adli, 2018).

Delivery of programmable nucleases

Safe and efficient delivery to target cells and tissues represents the most challenging aspect of successful gene therapy strategies. Accordingly, this challenge extends to genome-editing as well.

Programmable nucleases can be delivered in the form of DNA as plasmid or viral vectors, in-vitro transcribed mRNAs, or purified proteins. Unlike, DNA-based delivery, when nucleases are delivered as mRNA or proteins, they induce on-target mutations almost immediately after delivery inside cells and are degraded rapidly, reducing off-target effects without compromising on-target activity.

Generally, delivery systems are categorized into viral and nonviral vectors. The three most frequently used viral systems for in-vitro and ex-vivo genome-editing approaches are adenoviral vectors, adeno-associated

viral vectors (AAVs), and integrase-defective lentiviral vectors (a type of retroviral vector). However, in-vivo viral delivery depends mainly on the clinically approved AAVs (Wirth *et al.*, 2013). In-vivo gene delivery using AAVs to the eye, nervous system, liver, and skeletal and cardiac muscles has shown impressive efficacy in both preclinical models and clinical trials. However, its packaging capacity is limited to less than ~4.8 kb of DNA posing limitations for the delivery of large nucleases such as TALENs and SpCas9 nuclease (Yin *et al.*, 2017). A number of smaller Cas9 orthologs exist, and the ~3.1 kb Cas9 from *S. aureus* has been found to mediate highly efficient genome editing in-vivo after AAV delivery (Ran *et al.*, 2015). SpCas9 can also be divided into two lobes and reconstituted inside the transduced cells yielding a functional protein, however this approach may decrease the overall efficacy (Wright *et al.*, 2015).

On the contrary, nonviral delivery includes physical methods and nanomaterial-based vectors. Electroporation is the most widely utilized physical method. Clinical-grade electroporators have recently been developed (Derdelinckx *et al.*, 2016). Different synthetic lipid-based or polymer-based delivery vectors can also be used to transfect cells (Yin *et al.*, 2017).

Evaluating the activity of genome editing

Evaluating the efficacy of a nuclease platform involves analysis of both on-target and off-target activity. There are several methods that can be used to test the on-target changes (Hendel *et al.*, 2015; Koo *et al.*, 2015; Lee *et al.*, 2016). However, sequencing-based assays are the gold standard methods for evaluating the genome-editing outcomes, providing more sensitive and detailed information. On the contrary, it is more challenging to analyze the off-target activity. Generally, the off-target activity of a certain nuclease displays fixed location and frequency in a certain cell type and shows significant similarity among different cell types of certain species (Fu *et al.*, 2013). The off-target activity may result in cellular apoptosis or cell cycle arrest which can be detected over several days after nuclease delivery. Importantly, induction of extra DSBs is the major cause of nuclease cytotoxicity (Hendel *et al.*, 2015). To identify the off-target changes, a predictive technique will be applied to investigate locations prone to off-target activity. Then, potential targets will be analyzed using suitable enzyme or sequencing-based approach. In-vitro, in-cellula, and in-silico predictive techniques have been developed. However, the in-silico tools are most widely used owing to difficulty of the two other techniques (Tycko *et al.*, 2016). The most common off-target changes are small indels. However, the most concerning effects are gross chromosomal rearrangements as they are associated with cancerous transformation. Malignant transformation and rapid clonal expansion of cells treated with nuclease can be achieved without specifying the type and location of chromosomal rearrangement(s) (Porter *et al.*, 2014). However, to test particular rearrangement, specific PCR primers can be used (Mussolino *et al.*, 2014).

Ethical considerations

The extent to which applications of genome editing should be permitted raises several ethical issues throughout the global community (Brokowski and Adli, 2018). International organizations, professional bodies, and national academies of sciences and medicine raised the profile of genome editing by issuing statements on its appropriate uses. They favored somatic genome editing to treat or prevent a disease or disability rather than enhancement or less pressing purposes (e.g. somatic genome editing to increase musculature is permitted in a patient with muscular atrophy, but not in an individual with normal capabilities). Regulatory assessment of somatic genome editing encompasses minimization of risk, analysis of risks/benefits ratio, and investigating the procedure of participants' recruitment. Heritable germline editing denotes additional risk, because the alteration could affect descendants. Additionally, it could start us down a path towards non-therapeutic genetic enhancement. Most professional bodies and committees considered germline editing as a premature research field, owing to the associated issues of safety and efficacy are far from resolved and that attempts to apply this form of genome editing should not be made at this time. Human heritable genome-editing research trials are permitted for authorizing clinical trials only for compelling reasons and under strict oversight (National Academies of Sciences, Engineering, and Medicine, 2017). Human Genome Editing: Science, Ethics, and Governance, Washington, DC, the National Academies Press.

Clinical trials for therapeutic genome editing

At the therapeutic level, genome-editing approaches have been applied to provide effective strategies for treating a large array of human diseases and disorders including cancer, viral infections, cardiovascular diseases, inborn errors of metabolism, hematological disorders, muscular atrophy, and neurological disorders (Mollanoori and Teimourian, 2018). The potential of genome-editing preclinical settings enhances researchers to launch clinical trials. The first clinical trial was announced in USA for HIV. Currently, several trials for treating HIV, different types of malignancies, and monogenic disorders are underway (Table 2). With some clinical trials ongoing,

Table 2 Clinical trials of genome editing

Disease	Nuclease	Intervention	NCT number	Phase	First posted	Status	Countries	Available therapy
HIV	ZFN	CCR-5 disrupted CD4+ T cells	NCT00842634	1	12 February 2009	Completed	USA	ART
HIV	ZFN	CCR-5 disrupted CD4+ T cells	NCT01044654	1	8 January 2010	Completed	USA	
HIV	ZFN	CCR-5 disrupted CD4+ T cells	NCT01543152	1/2	2 March 2012	Completed	USA	
HIV	ZFN	CCR-5 disrupted CD4+ T cells	NCT02225665	1/2	26 August 2014	Active, not recruiting	USA	
HIV	ZFN	CCR-5 disrupted CD4+ T cells	NCT02388594	1	17 March 2015	Active, not recruiting	USA	
HIV	ZFN	CCR-5 disrupted CD34+ HSPCs	NCT02500849	1	17 July 2015	Recruiting	USA	
HIV	CRISPR/Cas9	Disrupted CCR-5 in CD34+ HSPCs	NCT03164135	Not applicable	23 May 2017	Recruiting	China	
HIV	ZFN	CCR-5 disrupted CAR-T cells	NCT03617198	1	6 August 2018	Not yet recruiting	USA	
Metastatic non-small-cell lung cancer	CRISPR/Cas9	PD-1 knockout T cells	NCT02793856	1	8 June 2016	Recruiting	China	Surgery, chemotherapy, radiotherapy
Metastatic advanced bladder cancer	CRISPR/Cas9	PD-1 knockout-T cells	NCT02863913	1	11 August 2016	Not yet recruiting	China	
Hormone refractory prostate cancer	CRISPR/Cas9	PD-1 knockout-T cells	NCT02867345	1	15 August 2016	Not yet recruiting	China	
Metastatic renal cell carcinoma	CRISPR/Cas9	PD-1 knockout-T cells	NCT02867332	1	15 August 2016	Not yet recruiting	China	
EBV positive advanced stage malignancies	CRISPR/Cas9	PD-1 knockout-T cells	NCT03044743	1/2	7 February 2017	Recruiting	China	
Esophageal cancer	CRISPR/Cas9	PD-1 knockout-T cells	NCT03081715	2	16 March 2017	Recruiting	China	
HPV-related cervical intraepithelial neoplasm	ZFN	Disrupted HPV16 and HPV18 E7 oncogene	NCT02800369	1	15 June 2016	Active, not recruiting	China	
HPV-related cervical intraepithelial neoplasm	TALEN CRISPR/Cas9	Disrupted HPV16 and HPV18 E6/E7 oncogenes	NCT03057912	1	20 February 2017	Not yet recruiting	China	
HPV-related cervical intraepithelial neoplasm	TALEN	Disrupted HPV16 E6/E7 oncogenes	NCT03226470	1	21 July 2017	Not yet recruiting	China	
B cell leukemia B cell lymphoma	CRISPR/Cas9	TCR and B2M knockout-CAR-T cells	NCT03166878	1/2	25 May 2017	Recruiting	China	
B-cell malignancies	CRISPR/Cas9	dual specificity CAR-T Cells	NCT03398967	1/2	16 January 2018	Recruiting	China	
Mesothelin positive multiple solid tumors	CRISPR/Cas9	PD-1 and TCR knockout-CAR-T cells	NCT03545815	1	4 June 2018	Recruiting	China	
Multiple myeloma Melanoma Synovial sarcoma Myxoid/round cell liposarcoma	CRISPR/Cas9	TCR and PD-1 knockout T-cells Engineered to Express NY-ESO-1 TCR	NCT03399448	1	16 January 2018	Recruiting	USA	

Contd...

Table 2 Contd...

Disease	Nuclease	Intervention	NCT number	Phase	First posted	Status	Countries	Available therapy
Metastatic gastrointestinal epithelial cancer	CRISPR/Cas9	CISH knockout-T cells	NCT03538613	1/2	28 May 2018	Not yet recruiting	USA	
Hemophilia B	ZFN	Factor IX transgene into the albumin locus in hepatocytes	NCT02695160	1	1 March 2016	Recruiting	USA	Concentrates of clotting factor IX
Transfusion-dependent β-thalassemia	ZFN	CD34+ HSPCs with disrupted erythroid-specific enhancer of the BCL11A gene	NCT03432364	1/2	14 February 2018	Recruiting	USA	Hydroxyurea or blood transfusion
Transfusion-dependent β-thalassemia	CRISPR/Cas9	CD34+HSPCs with disrupted erythroid-specific enhancer of the BCL11A gene	NCT03655678	1/2	31 August 2018	Recruiting	Germany	
MPS I	ZFN	IDUA transgene into the albumin locus in hepatocytes	NCT02702115	1	8 March 2016	Recruiting	USA	ERT
MPS II	ZFN	IDS transgene into the albumin locus in hepatocytes	NCT03041324	1	2 February 2017	Recruiting	USA	

All clinical trials of genome editing registered on ClinicalTrials.gov till 1 September 2018, were recruited.
ART, Antiretroviral therapy; BCL11A, B-cell lymphoma/leukemia 11A; CAR, chimeric antigen receptor; Cas9, clustered regularly interspaced short palindromic repeats-associated enzyme 9; CCR-5, C-C chemokine receptor type 5; CD, cluster of differentiation; CISH, cytokine-induced SH2 protein; CRISPR, clustered regularly interspaced short palindromic repeats; EBV, Epstein–Barr virus; ERT, enzyme replacement therapy; HPV, human papillomavirus; HSPCs, hematopoietic stem/progenitor cells; IDS, iduronate 2-sulfatase; IDUA, alpha-l-iduronidase; MPS, mucopolysaccharidosis; NCT number, ClinicalTrials.gov identifier; PD-1, programmed cell death protein 1; TALENs, transcription activator-like effector nucleases; ZFN, zinc finger nuclease.

genetic editing is much closer to being applied in the clinic. It is noteworthy that the available therapies of such diseases are not curative, usually require lifelong administration, and might exhibit individual variability to response. Moreover, they might cause a variety of adverse effects. This highlights the potential of the societal and economic effects if a genome editing-based therapy exchanges the conventional therapy. Most of these studies are still active and not completed yet. However, three studies for treating HIV have been completed supporting the feasibility of targeted genome editing using ZFNs to introduce a disease resistance allele and prevent HIV infection (Tebas *et al.*, 2014).

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