Application of Genome Editing Technologies for Disease Treatment: Review

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Authors’ contributions

This work was carried out in collaboration among all authors. Author GTB is the first and corresponding author designed the review and wrote the first draft of the manuscript. Authors PH and BAT managed the literature searches and critically revised the intellectual content. All authors read and approved the final manuscript.

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ABSTRACT

The improvement of particularly versatile genome-modifying advancements has outfitted experts with the ability to rapidly and monetarily bring sequence-specific changes into the genomes of a wide scope of cell types and organisms. The CRISPR framework was first found as a protection system in Escherichia coli against infections. Short portions of unfamiliar DNA are coordinated inside the CRISPR locus and translated into CRISPR RNA (crRNA), which at that point toughen to trans-activating crRNA (tracrRNA) to coordinate sequence specific debasement of pathogenic DNA by the Cas9 protein. Many studies have now revealed insight into the primary premise of DNA recognition by Cas9, showing that the heteroduplex shaped by the gRNA and its complementary strand of DNA is housed in a positively charged groove between the two nuclease areas (RuvC and HNH) inside the Cas9 protein, and that PAM recognition is intervened by an arginine-rich motif present in Cas9.

Genome altering biological tools likewise bring healing chances. For instance, ZFN-interceded gene interruption has been taken to the clinic, particularly for the treatment of glioblastoma and HIV by Sangamo biosciences. ZFNs focused to the HIV co-receptor CCR5 for the medication of...
HIV/AIDS are in stage I clinical trials have been finished currently and are in advancement). In these clinical investigations, the security and possibility of autologous infusion of ex vivo extended CD4+ T cells treated with CCR5-specific ZFNs are assessed in patients with HIV/AIDS. Genome altering itself likewise holds huge potential for treating the fundamental hereditary causes for specific infections. Thusly, the point of this survey is to sum up the vital standards of genome altering, focusing a considerable lot of the designing advances that have laid the foundation for the creation, refinement, and usage of the current set-up of genome-changing biological tools.

Keywords: Cleavage; CRISPR-Cas9; gRNA; TALENs; ZFNs.

1. INTRODUCTION

Few years back, the development of exceptionally adaptable genome-altering innovations has furnished specialists with the capacity to quickly and financially bring sequence-specific changes into the genomes of a wide range of cell types and organisms. The CRISPR system was first found as a defense system in Escherichia coli against viruses. This cutting edge innovation has the potential not exclusively to transformation and change the genetic pool in a general public, yet additionally to roll out essential improvements in the medical care framework, the food, medication, agriculture and all enterprises identified with natural sciences. Today, gene altering techniques are considered as new biological tools for research on disease treatment especially cancer. At first, two techniques for zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) were utilized for this reason [1]. These strategies had drawbacks because of significant expenses, the difficulty of endonucleases system design, and the low exhibition of exact cutting [2-4].

CRISPR was found in 1987 in E. coli. Actually, researchers found DNA sections which were successively rehashed at customary spans in the bacterial genome; however it required 20 years to turn out to be clear in 2007 that the rehashed sequences are indeed an acquired immune system in bacteria against viruses and plasmids [5]. As such, as the immune system of more intricate organic entities, similar to people, figures out how to manage germs and viruses when presented to them, bacteria additionally play out a comparative interaction utilizing CRISPR. Indeed, CRISPR ensures bacteria by the annihilation of the virus genome [6].

Hence, this system can possibly be utilized to change every gene from each of the 23 sets of human chromosomes with extraordinary precision, without initiating undesired mutations. Presently, CRISPR genome altering has become a molecular marvel for scientists, yet in addition for the entire world [7-9]. Thusly, the point of this survey is to sum up the vital standards of genome altering, focusing a considerable lot of the designing advances that have laid the foundation for the creation, refinement, and usage of the current set-up of genome-changing biological tools.

2. WHY THE ONLY HOPE IS TO USE GENOME EDITING APPROACH

Why CRISPR is advantageous than other gene editing biological tools? There are many reasons regarding why CRISPR-Cas9 is viewed as better than other gene editing tools. To begin with, it is a lot less expensive, more proficient, and more adaptable due to the specificity of its target DNA. Moreover, CRISPRs don’t should be combined with various, falsely made proteins to remove the piece of DNA since it secretes its own (Cas9). Addressing its adequacy, CRISPRs effectively match with the guide RNA (gRNA). This is conceivable in light of the fact that the gRNA is promptly accessible - a huge number of sequences are presented. Subsequently, the gRNA is likewise ready to focus on numerous genes and DNA sequences simultaneously.

3. CRISPR-Cas9

The CRISPR-Cas9 framework, which has a part in versatile immunity in bacteria [10-11], is the latest expansion to the genome-altering tool stash. In bacteria, the sort II CRISPR framework gives prevention against DNA from attacking viruses and plasmids through RNA-guided DNA cut by Cas proteins [12-13]. Short portions of unfamiliar DNA are coordinated inside the CRISPR locus and translated into CRISPR RNA (crRNA), which at that point toughen to trans-activating crRNA (tracrRNA) to coordinate sequence specific debasement of pathogenic DNA by the Cas9 protein [14]. In 2012, Charpentier, Doudna, and associates detailed
that target recognition by the Cas9 protein just needs a seed sequence inside the crRNA and a preserved protospacer-nearby motif (PAM) upstream of the crRNA joining site [14]. This framework has since been streamlined for genome designing [15-18] and now comprises of just the Cas9 nuclease and a solitary guide RNA (gRNA) contains the fundamental crRNA and tracrRNA components (Fig. 1).

From top to bottom: homing endonucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector (TALE) nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9). Homing endonucleases generally cleave their DNA substrates as dimers, and do not have distinct binding and cleavage domains. ZFNs recognize target sites that consist of two zinc-finger binding sites that flank a 5- to 7-base pair (bp) spacer sequence recognized by the FokI cleavage domain. TALENs recognize target sites that consist of two TALE DNA-binding sites that flank a 12- to 20-bp spacer sequence recognized by the FokI cleavage domain. The Cas9 nuclease is targeted to DNA sequences complementary to the targeting sequence within the single guide RNA (gRNA) located immediately upstream of a compatible protospacer adjacent motif (PAM). DNA and protein are not drawn to scale. [19].

Since target site recognition is interceded completely by the gRNA, CRISPR-Cas9 has arisen as the most adaptable and easy to understand stage for genome altering, wiping out the requirement for designing new proteins to perceive each new target site. The solitary significant restriction for Cas9 target site recognition is that the PAM motif which is perceived by the Cas9 nuclease and is fundamental for DNA split be found promptly downstream of the gRNA target site. The PAM sequence for the Streptococcus pyogenes Cas9, for instance, is 50-NGG-30 (albeit at times 50-NAG-30 can be endured) [20-22]. Many studies have now revealed insight into the primary premise of DNA recognition by Cas9, showing that the heteroduplex shaped by the gRNA and its complementary strand of DNA is housed in a positively charged groove between the two nuclease areas (RuvC and HNH) inside the Cas9 protein [23], and that PAM recognition is intervened by an arginine-rich motif present in Cas9 [24]. Doudna and partners have since recommended that DNA strand removal actuates a primary improvement inside the Cas9 protein that coordinates the no target DNA strand into the RuvC active site, which at that point positions the HNH space close to target DNA [25], empowering Cas9-intervened cleavage of both DNA strands.

Fig. 1. Genome-editing technologies. Cartoons illustrating the mechanisms of targeted nuclease
The Cas9 nuclease and its gRNA can be conveyed into cells for genome altering on the equivalent or separate plasmids, and various assets have been created to encourage target site choice and gRNA development, including E-CRISP [26], among others. In spite of the fact that Cas9 brags the most noteworthy simplicity use among the focused on nuclease stages, numerous reports have shown that it could be inclined to prompting off-target mutations [27,28].

To this end, significant exertion has been committed to improving the particularity of this system, including utilizing matched Cas9 nickases [22,29] which increment gene altering specificity by requiring the enlistment of two successive and contiguous nicking occasions for DSB development, or shortened gRNA that are more delicate to confound at the genomic target site than a full-length Grna [30]. Off-target split has likewise been diminished by controlling the dosage of either the Cas9 protein or Grna inside the cell [20], or even by utilizing Cas9 variations arranged to empower conditional genome altering, like a rapamycin inducible split-Cas9 design [31] or a Cas9 variation that contains a deliberately positioned little molecule responsive intein area [32].

Nucleofection [33] or transient transfection [34] of a preformed Cas9 ribonucleoprotein complex has additionally been appeared to decrease off target impacts, empowering without DNA free gene altering in essential human T cells [35], embryonic stem cells [36], Caenorhabditis elegans balls [37], mouse [38,39]; and zebrafish embryos [40], and even plant protoplasts [41].

The fuse of specific chemical modifications known to secure RNA from nuclease debasement and settle auxiliary design can additionally upgrade Cas9 ribonucleoprotein action ( [42,43]. In an astute marriage of genome-altering stages, the FokI split area has even been intertwined to an inactivated Cas9 variation to create hybrid nucleases that require protein dimerization for DNA split [44,45], hypothetically expanding CRISPRCas9 specificity. Likewise, combining Cas9 to DNA-joining areas has additionally demonstrated powerful at improving its particularity [46]. At last, many researches have currently showed that protein designing can extensively upgrade Cas9 particularity [47,48] and even adjust its PAM requirements [49], the last having the capacity to empower making of modified variations of Cas9 for allele-specific gene altering, despite the fact that Cas9 orthologs [16,49-52] or another CRISPR systems [53] with remarkable PAM specificities have been uncovered in nature.

4. GENOME EDITING TOOLS FOR GENE THERAPY

4.1 Targeted Disruption of Disease-Relevant Genes

Genome altering biological tools likewise bring healing chances. For instance, ZFN-interceded gene interruption has been taken to the clinic, particularly for the treatment of glioblastoma and HIV by Sangamo biosciences. In the previous case, the glucocorticoid receptor gene is disturbed by ZFNs in CD8+ cytotoxic T lymphocyte (CTL) as a feature of a T cell based cancer immunotherapy. These adjusted T-cells were demonstrated to have the option to obliterate glioblastoma tumor cells in animals in the presence of glucocorticoids. The clinical trial is right now in Phase I, assessing the wellbeing and bearableness of these designed T-cells [54] (NCT01082926).

ZFNs focused to the HIV co-receptor CCR5 for the medication of HIV/AIDS are in stage I clinical trials [55,56] (NCT01044654 has been finished as of late and NCT00842634 is in advancement). In these clinical investigations, the security and possibility of autologous infusion of ex vivo extended CD4+ T cells treated with CCR5-specific ZFNs are assessed in patients with HIV/AIDS. HIV disease needs the expression of co-receptors CCR5 or CXCR4. In the clinical trial, patient T cells are extricated and altered to communicate the mutant CCR5 allele which is impervious to HIV disease.

Another methodology a work in progress is to dispose of CCR5 in CD34+ HSCs with ZFNs which would permit the generation of CCR5-negative cells addressing all blood heredities. Contrasted with the other restorative methodologies (small molecular inhibitors, RNAi knockdown or impeding antibodies which need diligent exposure to the remedial), the expected favorable position of a ZFN approach is a completely penetrant and heritable gene knockout that endures for the lifetime of the cell and its offspring. TALENs have additionally been utilized to inactivate disease causing genes. A new report demonstrated the capability of TALENs for use in therapy of chronic hepatitis B infection (HBV) contamination. Some studies revealed that, the designed TALENs disturbed the episomal covalently close circular HBV DNA
5. Therapeutic Genome Editing

The ability to manipulate any genomic sequence by gene editing has created diverse opportunities to treating many various diseases and disorders (Fig. 2) [65]. Genome editing itself likewise holds huge potential for treating the fundamental hereditary causes for specific infections [66-68]. In quite possibly the best instances of this to date, ZFN-interceded interruption of the HIV co-receptor CCR5 was utilized to design HIV obstruction into both CD4+ T cells [69] and CD34+ hematopoietic stem/progenitor cells (HSPCs) [70], demonstrating protected and all around endured in a stage I clinical trial that imbued these gene-modified T cells into people with HIV/AIDS [71].

Besides empowering the presentation of gene adjustment that can upgrade autologous cell treatments, targeted nucleases can likewise be joined with viral vector involving AAV to intercede genome editing in situ [72]. For example, conveyance of an AAV vector encoding a ZFN pair intended to focus on a defective duplicate of the factor IX gene, alongside its maintenance format, prompted effective gene amendment in mouse liver, expanding factor IX protein production in both neonatal [73] and adult [74] models of the sickness. In vivo genome altering likewise lately empowered the reclamation of dystrophin gene articulation and the salvage of muscle work in mouse models of Duchenne muscular dystrophy [75-77].

Healing gene altering in a mouse model of human inherited tyrosinemia has likewise been accounted for utilizing both hydrodynamic infusion of plasmid DNA encoding CRISPRCas9 [78] and by consolidating nanoparticle-interceded conveyance of Cas9-encoding mRNA with AAV-intervened conveyance of the DNA layout for gene revision [79]. Currently, a double molecule AAV framework, wherein one AAV vector conveyed the Cas9 nuclease and a second held the gRNA and benefactor repair template, had the option to intervene revision of a sickness causing mutation in the ornithine transcarbamylase gene in the liver of a neonatal model of the disease [80].

6. CRISPR-MEDIATED LIVE CELL CHROMATIN IMAGING

The association of chromatin structure in the 3D nuclear space has a basic part in managing lineage-specific gene expression [81]. Truly, fluorescent in-situ hybridization (FISH) strategies have been principal in deciding the exact nuclear positions of specific genetic loci [82-84]. Besides that as it may, intrinsic constraints, like the prerequisite of cell fixation and sample warming,
disallowed the use of this technology to live cell imaging. Formerly, scientists utilized zinc fingers (ZNF) [85] and TALE proteins [86] for focused enrollment of fluorescent proteins to tedious genomic locales, for example, centromeres and telomeres for live cell imaging. Nonetheless, the advances in the dCas9 platform innovation have significantly improved both the effectiveness and extent of genome focusing for live cell chromatin imaging.

Fig. 2. Diversity of targets for therapeutic genome editing [65]
Scientists utilized fluorescently marked dCas9 to target redundant areas of the genome to accomplish the objective [87]. A comparative methodology has been used to target monotonous nature of telomeres and centromeres by co-articulation of dCas9 orthologs melded to various fluorescent proteins [88,89] and double color chromatin imaging of these redundant districts [89–92]. Focusing on dCas9 to a non-repetitive genomic locus is more difficult due to the foundation fluorescence signals because of free-skinning fluorescently labeled dCas9 proteins. Along these lines, transfection of as many as 26–36 unique sgRNAs is regularly needed to accomplish live cell imaging of a non-rehash genomic district [87,93].

To conquer this problem, researchers newly used designed sgRNA scaffolds which encompasses up to 16 MS2 binding modules to empower vigorous fluorescent signal enhancement and permit imaging a rehash genomic locale with as not many as 4 sgRNAs. The designed sgRNAs empowered multicolor naming of low-rehash containing districts utilizing a single sgRNA and of non-repetitive locales with as not many as four special sgRNAs. Prominently, this methodology empowered following of local chromatin loci all through the cell cycle and deciding differential situating of transcriptionally active and inactive regions in the 3D nuclear space [94].

Eminently, an exchange RNA adenosine deaminase has additionally been advanced and intertwined to nickase Cas9 to build up another novel base editor that accomplishes direct A→G change at the target locales [95]. These new base-altering approaches fundamentally extend the extent of genome focusing on. Specialists are further building up these techniques for extra purposes. We, and others, recently bound the proficiency of this CRISPR base editor to modify hereditary code and present early STOP codons in genes [98,99].

Researchers show that by altering C into T at CGA (Arg), CAG (Gln), and CAA (Gln) codons, scientists can make TGA (opal), TAG (golden), or TAA (ochre) STOP codons, separately. The CRISPR-STOP approach is a productive and less pernicious option in contrast to WTCas9-intervened gene knockout (KO) studies [99]. Notwithstanding the APOBEC adenosine deaminase enzyme, the actuation instigated adenosine deaminase (AID) enzyme has likewise been combined to the dCas9 enzyme [100,101]. Eminently, without UGI in the complex the dCas9–AID complex turns into an incredible neighborhood mutagenic agent that goes about as an addition of capacity screening tool] [100–102].

8. CRISPR-MEDIATED EPIGENOME EDITING

The meaning of "epigenetics" is intensely discussed. Here, researchers utilize "epigenetic" to infer the molecular mechanism of heritable gene expression changes that can't be ascribed to changes in DNA sequence data. Dissimilar to epigenetics, which suggests the mechanism, the epigenome portrays all posttranslational changes and other chromatin highlights related with regulatory components in the genome. Ongoing large-scale epigenomic endeavors like the Encyclopedia of DNA components (ENCODE) and Roadmap Epigenome Mapping Consortium (REMC) endeavors have mapped chromatin adjustments both on DNA and histone proteins across the genome in different cell lines just as essential cell types and tissues [103,104].

Albeit these epigenomic maps uncovered uncommon understanding into cell-type specific gene guideline and genome organization, the useful parts of different epigenomic highlights, like histone changes and DNA methylation, stay to be completely perceived. To this end, locus-specific epigenome planning tools and advances
are relied upon to significantly enable scientists to explain useful jobs of chromatin changes. Such types of biological tools will empower researchers to probe the long-standing inquiries of chromatin biology, for example, the causal connection between the presence of an epigenetic mark and gene articulation [105,106].

Besides, the capacity the change locus-specific epigenetic marks may empower us to distinguish temporal kinetics of an epigenetic mark and its actual part on the useful epigenetic memory and quality articulation. Hence, soon after the CRISPR-Cas9 framework was bound as an effective gene altering innovation, analysts utilized the programmable capacity of dCas9 to enroll different epigenetic writers and erasers to a specific locus. There are various layers of epigenetic regulatory mechanisms working in the genome. Among the all-around depicted ones are DNA methylation, histone posttranslational alterations, and non-coding RNAs (short and long). Among these, DNA methylation has the longest history, as scientists saw and began to examine its function in gene articulation and advancement in the early 1970s [107,108].

DNA methylation is quite possibly the most generally considered epigenetic mechanisms of gene editing guideline. Outstandingly, in plants and different organisms, DNA methylation is found in three diverse arrangement settings: CG (or CpG), CHG, or CHH (H is A, T, or C), though in mammalian systems, most of DNA methylation occurs at the fifth carbon of Cytosine residues (5-methylcytosine) of CpG dinucleotides [109]. DNMT3A and DNMT3B are the two DNA methyltransferase enzymes that catalyze de novo DNA methylation [110].

5-Cytosine DNA methylation at adviser or distal administrative components is for the most part connected with transcriptional restraint. Variant DNA methylation has been ensnared in various pathological diseases including malignant tumor. In this manner, there is strong unmet remedial need to control atypical sickness related epigenomic highlights. In accordance with this, a portion of molecule epigenetic inhibitors that worldwide target DNA methylation such as 5-azacytidine are FDA approved [111].

Though such small molecules are now in clinical use, they focus on the whole genome and in this way modify the chromatin condition of loci where the epigenetic state is normal. Subsequently, creating locus-specific epigenetic altering biological tools that specifically target aberrantly regulated loci has extraordinary restorative potential. To accomplish this confirmation of head, scientists overlaid dCas9 to catalytic domain of eukaryotic DNA methyl transferase (DNMT3A) [112–119] or prokaryotic DNA methyltransferase (MQ3) [116].

9. LARGE-SCALE GENETIC AND EPIGENETIC CRISPR SCREENINGS

Notwithstanding focused on hereditary and epigenetic controls, the straightforward and proficient gene focusing on capacity of CRISPR has been bridled to accomplish enormous scope utilitarian screenings. In such applications, rather than utilizing a solitary sgRNA, WT Cas9 or dCas9-effector combination proteins are guided with hundreds or thousands of each sgRNAs in a populace of cells. A definitive focus on such investigations is to recognize genes that impact a particular phenotype in an unbiased fashion [120]. Albeit the methodology requires various specialized and scientific contemplations, when set up, such a methodology turns into an amazing high throughput assay to practically screen countless genes simultaneously. In its fundamental structure, a huge pool of Cas9/sgRNAs are commonly conveyed to a populace of cells through a low variety of viral disease (MOI = 0.3 to 0.4). This guarantees that every cell is accepting one or less sgRNA. For powerful measurable readouts, every gene is ordinarily focused by 6–10 diverse sgRNAs.

The fundamental rationale behind the CRISPR KO screenings is that if a gene is fundamental for a given phenotype, like cell multiplication, at that point the cells infected with the sgRNAs focusing on that gene will be moderately exhausted from the populace over the long run. Since each sgRNA is steadily coordinated into the genome during viral contamination, the controlling sequences of each sgRNA can be utilized as a special ‘barcode’. In this way the general plenitude of each sgRNA in a given populace of cells can be measured by targeted sequencing. The particular subtleties of such measures are past the extent of this survey. [121-123].

10. POTENTIAL CHALLENGES

Albeit the capacities of CRISPR/Cas9 framework are plainly settled and have been utilized in
different applications, there are concerns in regards to off-target changes, which may restrict its future viewpoints. Information from many investigations shows that the off-target impacts of the CRISPR/Cas9 framework are among the main outcomes of this technique, paying little mind to the cell type and target genes [124–129]. Hybrid R-loop formation between sgRNA and the focused DNA may result in double-stranded cleavage of DNA because of RNA-guided nucleases, the acknowledgment of PAM sequences and the presence of nearby AAMs [130].

Moreover, it was shown that such action brings about an expanded degree and a high volume of off-target impacts by CRISPR/Cas9 during gene treatment, particularly because of dsDNA break and NHEJ work [131]. Different procedures and conventions have been intended to enhance the low specificity of CRISPR/Cas9 and to advance HDR-based repair over NHEJ, to decrease the mutation rate. Openness of little circle-iPSCs to cold stun or low temperatures after treatment with CRISPR/Cas framework brought about expanded HDR capacity and, accordingly, diminished off-target impacts. Notwithstanding, the rate of indel arrangement was not significantly influenced [132].

Another research intended to decrease the off-target impacts explored changing the proportion of sgRNA to Cas9 protein, and showed that a higher proportion of sgRNA to Cas9 brought about diminished frequency of off-target impacts [130]. Choice of bacteria for reaping Cas9 uniquely influences the presentation of CRISPR/Cas9. For instance, many researches explored the effect of the CRISPR/Cas9 framework utilizing three distinct types of microorganisms; namely, Streptococcus pyogenes Cas9 (SpCas9), S. thermophilus Cas9 (St1Cas9) and SaCas9 [130,133]. The assessment of human cells transfected with Cas9 plasmids from bacteria showed extended activity, similarly as reduced mutation rates, differentiated and SpCas9 and SaCas9 [134]. Despite the disclosures referred to over, the base sequence of the AAM upstream of PAM plays a crucial part in sgRNA binding with proto spacers on the target DNA [135]. sgRNAs with a higher extent of guanine and a lower extent of adenine are all the more consistent in binding with target DNA compared with sgRNAs from a higher extent of cytosine [136]. Various challenges consolidate plasmids with low specificity and random integration into the target DNA, which makes tracking obstacles [130].

11. FUTURE DIRECTIONS

Improvement of novel instruments and advances is essential for logical headway. Nobel laureate Sydney Brenner is cited as saying "Progress in science relies upon new strategies, new disclosures and groundbreaking thoughts, most likely in a specific order" [137]. Doubtlessly, CRISPR-based advancements have enabled scientists with a phenomenal toolbox. The history of molecular biology will put CRISPR-Cas9 among the significant biological tools that empowered advancement disclosures and methodological progressions in science.

CRISPR applications have effectively extended our vision of genome guideline and association in living cells across different organic realms. In such manner, CRISPR isn’t just changing molecular biology yet in addition medicine and biotechnology. Because of space constraints, this survey just centered around the major CRISPR tools. In any case, various ongoing survey articles have thoroughly outlined the particular utilizations of CRISPR technology [138,139–145]. Within the last few years researchers have seen stunning advancement in the improvement of different CRISPR-based advances. The clinical utilizations of the CRISPR innovations are especially energizing [146].

Such progressions have been broadly shrouded in friendly and other broad communications outlets, motivating incredible fervor and interest from the overall population. Nonetheless, the quick advancement of CRISPR-based apparatuses likewise delivers various specialized difficulties alongside friendly and moral concerns. One of the specialized difficulties is the conveyance of such apparatuses into living cells and organisms. Scientists normally utilize viral vectors to convey genes of interest in vivo or in vitro. Because of their low immunogenicity, AAV vectors are especially alluring helpful conveyance vehicles for in vivo settings. Though, the huge size of current Cas proteins makes a significant problem in their packaging into AAV vectors.

In this manner, future headways in lessening the size of existing Cas proteins or the revelation of more modest Cas9 proteins is exceptionally required. As CRISPR advancements fill in degree and force, social and moral worries over their utilization are likewise rising, and uses of
accomplished, numerous difficulties stay befo
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ahead into clinical trials, this factor should be
considered [153]. Examining and seeing such
difficulties will empower us to all the more likely
decide the extent of their impediments and
approaches to defeat them. To this end, one
proposed answer for the immunogenicity issue
could be to recognize and use orthogonal
CRISPR-Cas9 proteins to which we as humans
have not been introduced before [154]. Almost
certainly, a lot more naturally happening CRISPR
frameworks will be found and that they will be
attached for extra genome-targeting platforms. In
this way, in corresponding to the current
progressions, extra researches are expected to
address the wellbeing and explicitness of such
biological tools. Besides, adequate
contemplations should be dedicated to the social
and moral ramifications of such innovations so
they will be open to all layers of society and
advantage all humanity.

12. CONCLUSIONS
Regardless of the triumphs previously
accomplished, numerous difficulties stay before
the maximum capacity of genome editing can be
figured it out. Most importantly are the
advancement of new devices fit for presenting
genomic adjustments without DNA breaks.
Directed recombinases which can be customized
to perceive specific DNA sequences and even
coordinate remedial components into the human
gene, are one such alternative. Later work
has shown that solitary base editing without DNA
breaks can be accomplished utilizing a designed
Cas9 nickase complex, in spite of the fact that it
stays obscure how compelling this innovation is
in therapeutically important settings.

By connecting genomic changes actuated by
focused nuclease to their own self degradation,
self-inactivating vectors are additionally ready to
improve the particularity of genome editing,
particularly on the grounds that the frequency of
off-target adjustments can be straightforwardly
corresponding to the duration of cellular
exposure to a nuclease. What's more, a
significant part of the information behind genome
designing has been acquired in immortalized cell
lines Nevertheless, on account of regenerative
medicine; it is profoundly alluring to genetically
manipulate progenitor or stem-cell populations,
the two of which can contrast extraordinarily from
transformed cell lines as for their epigenome or
three-dimensional association of their genomic
DNA.

Tremendous progress has been made in
addressing the challenges of conventional gene
therapy by developing new technologies for
precise modification of the human genome. This
has helped to overcome some of the obstacles
that have plagued the field of gene therapy for
decades. Nevertheless, many challenges still
remain to fully realize the potential of genome
editing for gene and cell therapy. Central to these
challenges are the persistent issues of safety
and delivery. In this regard, rapid advances are
being made both for increasing the specificity of
genome-editing tools and increasing the
sensitivity of methods for assessing this
specificity genome-wide. However, it remains
unclear whether all off-target effects can be
accounted for in a therapy that targets one site
within billions of DNA base pairs, involves
modification of millions of cells, and is custom
prepared for each patient.

Besides, numerous inquiries stay about how the
human immune system will react to hereditarily
altered cells or the in vivo administration of
genome-altering tools. Wonderful progression in
delivery technologies are likewise setting out a lot more open doors for genome altering, including ex vivo conveyance to cells with without DNA-free free components and in vivo conveyance with productive and tissue-specific vectors. The numerous triumphs of the preclinical examinations evaluated here, just as the current movement of genome altering in clinical trials, is a wellspring of huge confidence for the fate of this field.

The fast advancement in the field is probably going to keep on prompting new innovations that will extend the extent of genome altering. Elective genome-altering advances, for example, targetable site-specific recombinases that don't depend on the formation of twofold strand breaks, elective CRISPR frameworks with interesting properties, and DNA-guided nuclease frameworks will keep on changing what is conceivable with these biological tools.

Epigenome altering, in which DNA-focusing on stages are utilized to explicitly change gene regulation or chromatin structure, is likewise making better approaches to control the genome for gene and cell treatment. Inducible or self-regulating systems those empower the control of the articulation, action, as well as solidness of genome-altering tools may assume a significant part in guaranteeing their accuracy and wellbeing. In summary, genome altering has changed the meaning of gene and cell treatment and has been a vital factor in the new resurgence of this field, but there is still critical key and translational work to understand the full guarantee of these advancements for generally treating human disease.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Carroll D. Genome editing: progress and challenges for medical applications. Genome Med. 2016;8(1):120.
2. Sanchez-Rivera FJ, Jacks T. Applications of the CRISPRCas9 system in cancer biology. Nat Rev Cancer. 2015;15(7):387–395.
3. Sachdeva M, Sachdeva N, Pal M, et al. CRISPR/Cas9: molecular tool for gene therapy to target genome and epigenome in the treatment of lung cancer. Cancer Gene Ther. 2015;22(11):509–517.
4. Yao S, He Z, Chen C. CRISPR/Cas9-mediated genome editing of epigenetic factors for cancer therapy. Hum Gene Ther. 2015;26(7):463-471.
5. Marraffini LA, Sontheimer EJ. CRISPR interference: RNA-directed adaptive immunity in bacteria and archaea. Nat Rev Genet. 2010;11(3):181-190.
6. Rath D, Amlinger L, Rath A, et al. The CRISPR-Cas immune system: biology, mechanisms and applications. Biochimie. 2015;117:119-128.
7. Sorek R, Kunin V, Hugenholtz P. CRISPR—a widespread system that provides acquired resistance against phages in bacteria and archaea. Nat Rev Microbiol. 2008;6(3):181–186.
8. Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014;346(6213):1258096.
9. Kim EJ, Kang KH, Ju JH. CRISPR-Cas9: A promising tool for gene editing on induced pluripotent stem cells. Korean J Intern Med. 2017;32(1):42–61.
10. Horvath P, Barrangou R. CRISPR/Cas, the immune system of bacteria and archaea. Science. 2010;327:167–170.
11. Marraffini LA, Sontheimer EJ. CRISPR interference: RNA-directed adaptive immunity in bacteria and archaea. Nat Rev Genet 2010;11:181-190.
12. Wiedenheft B, Sternberg SH, Doudna JA. RNA-guided genetic silencing systems in bacteria and archaea. Nature. 2012;482:331-338.
13. Sorek R, Lawrence CM, Wiedenheft B. CRISPR-mediated adaptive immune systems in bacteria and archaea. Annu Rev Biochem 2013;82:237–266.
14. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA
Methods

Fast CRISPR target site identification. Nat Biotechnol. 2013;31:230–232.

Cho SW, Kim S, Kim JM, Kim JS. Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. Nat Biotechnol. 2013;31:230–232.

Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, et al. 2013. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013;339:819-823.

Jinek M, East A, Cheng A, Lin S, Ma E, Doudna J. RNA-programmed genome editing in human cells. Elife. 2013;2:e00471.

Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, et al. RNA-guided human genome engineering via Cas9. Science. 2013;339:823–826.

Gaj T, Sirk SJ, Shui SL, Liu J. Genome-editing technologies: Principles and applications. Cold Spring Harbor Perspectives in Biology. 2016;8(12):a023754.

Hsu PD, Scott DA, Weinstein JA, Ran FA, Konermann S, Agarwala V, et al. DNAtargeting specificity ofRNA-guided Cas9 nucleases. Nat Biotechnol 2013;31:827–832.

Jiang W, Bikard D, Cox D, Zhang F, Marraffini LA. RNA-guided editing of bacterial genomes using CRISPRCas systems. Nat Biotechnol 2013;31:233–239.

Mali P, Aach J, Stranges PB, Esvelt KM, Moosburner M, Kosuri S, Yang L, Church GM. 2013a. CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. Nat Biotechnol 2013;31:833-838.

Nishimasu H, Ran FA, Hsu PD, Konermann S, Shehata SI, Dohmae N, et al. Crystal structure of Cas9 in complex with guide RNA and target DNA. Cell. 2014;156:935-949.

Anders C, Niewoehner O, Duerst A, Jinek M. Structural basis of PAM-dependent target DNA recognition by the Cas9 endonuclease. Nature. 2014;513:569–573.

Jiang F, Taylor DW, Chen JS, Kornfeld JE, Zhou K, Thompson AJ, et al. Structures of a CRISPR-Cas9 R-loop complex primed for DNA cleavage. Science. 2016;351:867–871.

Heigwer F, Kerr G, Boutros M. E-CRISP: Fast CRISPR target site identification. Nat Methods 2014;11:122–123.

Cradick TJ, Fine EJ, Antico CJ, Bao G. 2013. CRISPR/Cas9 systems targeting b-globin and CCR5 genes have substantial off-target activity. Nucleic Acids Res 2013;41:9584-9592.

Fu Y, Foden JA, Khayter C, Maeder ML, Reyon D, Joung JK, et al. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. Nat Biotechnol. 2013;31:822–826.

Ran FA, Hsu PD, Lin CY, Gootenberg JS, Konermann S, Trevino AE, et al. Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. Cell 2013;154:1380-1389.

Fu Y, Sander JD, Reyon D, Cascio VM, Joung JK. 2014. Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. Nat Biotechnol 2014;32:279-284.

Zetsche B, Volz SE, Zhang F. A split-Cas9 architecture for inducible genome editing and transcription modulation. Nat Biotechnol. 2015;33:139–142.

Davis KM, Pattanayak V, Thompson DB, Zuris JA, Liu DR. Small molecule-triggered Cas9 protein with improved genome-editing specificity. Nat Chem Biol 2015;11:316–318.

Kim S, Kim D, Cho SW, Kim J, Kim JS. Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. Genome Res 2014;24:1012–1019.

Zuris JA, Thompson DB, Shu Y, Guilinger JP, Bessen JL, Hu JH, et al. Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing in vitro and in vivo. Nat Biotechnol. 2015;33:73–80.

Schumann K, Lin S, Boyer E, Simeonov DR, Subramaniam M, Gate RE, et al. 2015. Generation of knock-in primary human T cells using Cas9 ribonucleoproteins. Proc Natl Acad Sci. 2015;112:10437–10442.

Liu J, Gaj T, Yang Y, Wang N, Shui S, Kim S, et al. Efficient delivery of nuclease proteins for genome editing in human stem cells and primary cells. Nat Protoc 2015;10:1842–1859.

Paix A, Folkmann A, Rasoloson D, Seydoux G. High efficiency, homology-directed genome editing in Caenorhabditis elegans using CRISPR-Cas9 ribonucleo protein complexes. Genetics. 2015;201:47–54.
38. Menoret S, De Cian A, Tesson L, Remy S, Usal C, Boule JB, et al. Homology-directed repair in rodent zygotes using Cas9 and TALEN engineered proteins. Sci Rep. 2015;5:14410.

39. Wang L, Shao Y, Guan Y, Li L, Wu L, Chen F, et al. Large genomic fragment deletion and functional gene cassette knock-in via Cas9 protein mediated genome editing in one-cell rodent embryos. Sci Rep. 2015;5:17517.

40. Sung YH, Kim JM, Kim HT, Lee J, Jeon J, Jin Y, et al. Highly efficient gene knockout in mice and zebrafish with RNA-guided endonucleases. Genome Res 2014;24:125-131.

41. Woo JW, Kim J, Kwon SI, Corvalan C, Cho SW, Kim H, et al. 2015. DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. Nat Biotechnol. 2015;33:1162–1164.

42. Hendel A, Bak RO, Clark JT, Kennedy AB, Ryan DE, Roy S, et al. Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells. Nat Biotechnol 2015;33:985–989.

43. Rahdar M, McMahon MA, Prakash TP, Swayne EE, Bennett CF, Cleveland DW. Synthetic CRISPR RNA-Cas9- guided genome editing in human cells. Proc Natl Acad Sci 2015;112:E7110–E7117.

44. Guilinger JP, Thompson DB, Liu DR. Fusion of catalytically inactive Cas9 to FokI nuclease improves the specificity of genome modification. Nat Biotechnol 2014;32:577–582.

45. Tsai SQ, Wyvekens N, Khyter C, Foden JA, Thapar V, Reyon D, et al. Dimeric CRISPR RNA-guided FokI nucleases for highly specific genome editing. Nat Biotechnol 2014;32:569–576.

46. Bolukbasi MF, Gupta A, Oikemus S, Derr AG, Garber M, Brodsky MH, et al. DNA-bindingdomain fusions enhance the targeting range and precision of Cas9. Nat Methods 2015;12:1150–1156.

47. Kleinstiver BP, Pattanayak V, Prew MS, Tsai SQ, Nguyen NT, Zheng Z, et al. High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. Nature 2016;529:490–495.

48. Slaymaker IM, Gao L, Zetsche B, Scott DA, Yan WX, Zhang F. Rationally engineered Cas9 nucleases with improved specificity. Science 2016;351:84–88.

49. Kleinstiver BP, Prew MS, Tsai SQ, Topkar VV, Nguyen NT, Zheng Z, et al. Engineered CRISPR-Cas9 nucleases with altered PAM specificities. Nature. 2015;523:481-485.

50. Esvelt KM, Mali P, Braff JL, Moosburner M, Yang SJ, Church GM. 2013. Orthogonal Cas9 proteins for RNA-guided gene regulation and editing. Nat Methods. 2013;10:1116-1121.

51. Hou Z, Zhang Y, Propson NE, Howden SE, Chu LF, Songheimer EJ, et al. 2013. Efficient genome engineering in human pluripotent stem cells using Cas9 from Neisseria meningitidis. Proc Natl Acad Sci. 2013;110:15644-15649.

52. Ran FA, Cong L, Yan WX, Scott DA, Gootenberg JS, Kriz AJ, et al. ln vivo genome editing using Streptococcus aureus Cas9. Nature. 2015;520:186–191.

53. Zetsche B, Gootenberg JS, Abudayeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell. 2015;163:759–771.

54. Reik A, Zhou Y, Hamett A, et al. Zinc Finger Nucleases Targeting the Glucocorticoid Receptor Allow IL-13 Zetakine Transgenic CTLs To Kill Glioblastoma Cells In vivo in the Presence of Immunosuppressing Glucocorticoids. Mol Ther. 2008;16:S13-S14.

55. Holt N, Wang J, Kim K, et al. Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 In vivo. Nat Biotechnol. 2010; 28:839-47.

56. Perez EE, Wang J, Miller JC, et al. Establishment of HIV-1 resistance in CD4+ T cells by genome editing using zinc-finger nucleases. Nat Biotechnol 2008; 26:808-16.

57. Bloom K, Ely A, Mussolino C, Cathomen T, Arbuthnot P. Inactivation of Hepatitis B Virus Replication in Cultured Cells and In vivo with Engineered Transcription Activator-Like Effector Nucleases. Mol Ther. 2013;21(10):1889-97. DOI: 10.1038/mt.2013.170.

58. Urnov FD, Miller JC, Lee YL, et al. Highly efficient endogenous human gene correction designed zinc-finger nucleases. Nature. 2005;435:646-51.

59. Soldner F, Laganiere J, Cheng AW, et al. Generation of isogenic pluripotent stem cells differing exclusively at two early onset Parkinson point mutations. Cell 2011;146:318-31.
60. Yusa K, Rashid ST, Strick Marchand H, et al. Targeted gene correction of alpha1-antitrypsin deficiency in induced pluripotent stem cells. Nature. 2011;478:391-4.

61. Sun N, Zhao H. Seamless correction of the sickle cell disease mutation of the HBB gene in human induced pluripotent stem cells using TALENs. Biotechnol Bioeng. 2013. DOI: 10.1002/bit.25018.

62. Zou J, Sweeney CL, Chou BK, et al. Oxidase-deficient neutrophils from X-linked chronic granulomatous disease iPSC cells: functional correction by zinc finger nuclease-mediated safe harbor targeting. Blood. 2011; 117:5561-72.

63. Chang CJ, Bouhassira EE. Zinc-finger nuclease-mediated correction of alpha-thalassemia in iPSC cells. Blood 2012; 120:3906-14.

64. Li H, Haurigot V, Doyon Y, et al. In vivo genome editing restores haemostasis in a mouse model of haemophilia. Nature. 2011;475:217-21

65. Maeder ML, Gersbach CA, Genome-editing technologies for gene and cell therapy. Molecular Therapy. 2016;24(3):430-446.

66. Cox DB, Platt RJ, Zhang F. Therapeutic genome editing: Prospects and challenges. Nat Med. 2015;21:121–131.

67. Porteus MH. Towards a new era in medicine: Therapeutic genome editing. Genome Biol 2015;16: 286.

68. Maeder ML, Gersbach CA. Genome-editing technologies for gene and cell therapy. Mol Ther 2016;24:430-446.

69. Perez EE, Wang J, Miller JC, Jouvenot Y, Kim KA, Liu O, et al. Establishment of HIV-1 resistance in CD4+ T cells by genome editing using zinc-finger nucleases. Nat Biotechnol 2008;26:808–816.

70. Holt N, Wang J, Kim K, Friedman G, Wang X, Taupin V, et al. Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 in vivo. Nat Biotechnol. 2010;28: 839–847.

71. Tebas P, Stein D, Tang WW, Frank I, Wang SQ, Lee G, et al. Gene editing of CCR5 in autologous CD4+ T cells of persons infected with HIV. N Engl J Med. 2014;370:901–910.

72. Gaj T, Epstein BE, Schaffer DV. Genome engineering using adeno-associated virus: Basic and clinical research applications. Mol Ther. 2016;24:458-464.

73. Li H, Haurigot V, Doyon Y, Li T, Wong SY, Bhagwat AS, et al. In vivo genome editing restores haemostasis in a mouse model of haemophilia. Nature. 2011;475:217–221.

74. Anguela XM, Sharma R, Doyon Y, Miller JC, Li H, Haurigot V, et al. Robust ZFN-mediated genome editing in adult hemophilic mice. Blood. 2013;122:3283–3287.

75. Long C, Amoasii L, Mireault AA, McAnally JR, Li H, Sanchez-Ortiz E, et al. Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. Science 2015;351:400–403.

76. Nelson CE, Hakim CH, Ousterout DG, Thakore PI, Moreb EA, Rivera RMC, et al. In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. Science. 2015;351:403–407.

77. Tabebordbar M, Zhu K, Cheng JK, Chew WL, Widrick JJ, Yan WX, et al. In vivo gene editing in dystrophic mouse muscle and muscle stem cells. Science. 2015;351:407–411.

78. Yin H, Xue W, Chen S, Bogorad R, Benedetti E, Grompe M, Koteliantsky V, et al. Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. Nat Biotechnol. 2014;32:551–553.

79. Yin H, Song CQ, Dorkin JR, Zhu LJ, Li Y, Wu Q, et al. Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo. Nat Biotechnol. 2016;34:328–333.

80. Yang Y, Wang L, Bell P, McMenamin D, He Z, White J, Yu H, et al. A dual AAV system enables the Cas9-mediated correction of a metabolic liver disease in newborn mice. Nat Biotechnol. 2016;34: 334–338.

81. Dixon JR et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature. 2012; 485:376-380.

82. Bickmore WA. The spatial organization of the human genome. Annu. Rev. Genomics Hum. Genet. 2013;14:67–84.

83. Meaburn KJ, Misteli T, Soutoglou E. Spatial genome organization in the formation of chromosomal translocations. Semin. Cancer Biol. 2007;17:80–90.

84. Shachar S, Voss TC, Pegoraro G, Sciascia N, Misteli T. Identification of gene
positioning factors using high-throughput imaging mapping. Cell. 2015;162:911–923.
85. Lindhout BI et al. Live cell imaging of repetitive DNA sequences via GFP-tagged polydactyl zinc finger proteins. Nucleic Acids Res. 2007;35:e107.
86. Miyanari Y, Ziegler Birling C, Torres Padilla ME. Live visualization of chromatin dynamics with fluorescent TALEs. Nat. Struct. Mol. Biol. 2013;20:1321–1324.
87. Chen B et al. Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas system. Cell. 2013;155:1479–1491.
88. Ma H et al. Multicolor CRISPR labeling of chromosomal loci in human cells. Proc. Natl Acad. Sci. USA 2015;112:3002–3007.
89. Shao S et al. Long-term dual-color tracking of genomic loci by modified sgRNAs of the CRISPR/Cas9 system. Nucleic Acids Res. 2016;44:e86.
90. Wang S, Su JH, Zhang F, Zhuang X. An RNA-aptamer-based two-color CRISPR labeling system. Sci. Rep. 2016;6:26857.
91. Fu Y et al. CRISPR-dCas9 and sgRNA scaffolds enable dual-colour live imaging of satellite sequences and repeat-enriched individual loci. Nat. Commun. 2016;7:11707.
92. Ma H et al. Multiplexed labeling of genomic loci with dCas9 and engineered sgRNAs using CRISPRRainbow. Nat. Biotechnol. 2016;34:528–530.
93. Anton T, Bulthmann S, Leonhardt H, Markaki Y. Visualization of specific DNA sequences in living mouse embryonic stem cells with a programmable fluorescent CRISPR/Cas system. Nucleus 2014;5:163–172.
94. Qin P et al. Live cell imaging of low- and non-repetitive chromosome loci using CRISPR-Cas9. Nat. Commun. 2017;8:14725.
95. Gaudelli NM et al. Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage. Nature. 2017;551:464–471.
96. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without doublestranded DNA cleavage. Nature. 2016;533:420–424.
97. Nishida K et al. Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems. Science. 2016;353.
98. Kuscu C et al. CRISPR-STOP: Gene silencing through base editing-induced nonsense mutations. Nat. Methods. 2017;14:710–712.
99. Billon P et al. CRISPR-mediated base editing enables efficient disruption of eukaryotic genes through induction of STOP codons. Mol. Cell. 2017;67:1068–1079.e1064.
100. Hess GT et al. Directed evolution using dCas9-targeted somatic hypermutation in mammalian cells. Nat. Methods. 2016;13:1036-1042.
101. Ma Y et al. Targeted AID-mediated mutagenesis (TAM) enables efficient genomic diversification in mammalian cells. Nat. Methods 2016;13:1029–1035.
102. Kuscu C, Adli, M. CRISPR-Cas9-AID base editor is a powerful gain-offunction screening tool. Nat. Methods 2016;13:983–984.
103. Ptashne M. On the use of the word ‘epigenetic’. Curr. Biol. 2007;17:R233–R236.
104. Greally JM. A user’s guide to the ambiguous word ‘epigenetics’. Nat. Rev. Mol. Cell. Biol. 2018;19:207–208.
105. Consortium EP et al. An integrated encyclopedia of DNA elements in the human genome. Nature. 2012;489:1042–1048.
106. Bernstein, B. E. et al. The NIH Roadmap Epigenomics Mapping Consortium. Nat. Biotechnol. 2010;2:1045–1048.
107. Holliday R, Pugh JE. DNA modification mechanisms and gene activity during development. Science. 1975;187:226–232.
108. Riggs AD. X inactivation, differentiation, and DNA methylation. Cytogenet. Cell Genet. 1975;14:9–25.
109. Razin A, Riggs AD. DNA methylation and gene function. Science. 1980;210:604–610.
110. Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell. 1999;99:247–257.
111. Kaminskas E, Farrell AT, Wang YC, Sridhara R, Pazdur R. FDA drug approval summary: azacitidine (5-azacytidine, Vidaza) for injectable suspension. Oncologist. 2005;10:176–182.
112. Liu, X. S. et al. Editing DNA methylation in the mammalian genome. Cell 2016;167:233–247.e17.
113. Amabile A et al. Inheritable silencing of endogenous genes by hit-and-run targeted epigenetic editing. Cell. 2016;167:219–232.e214.
114. Vogta, A. et al. Repurposing the CRISPR-Cas9 system for targeted DNA methylation. Nucleic Acids Res. 2016;44:5615–5628.
115. Mc Donald, JI. et al. Reprogrammable CRISPR/Cas9-based system for inducing site-specific DNA methylation. Biol. Open 2016;5:866–874.
116. Lei Y et al. Targeted DNA methylation in vivo using an engineered dCas9–MO1 fusion protein. Nat. Commun. 2017;8:16026.
117. Xiong T et al. Targeted DNA methylation in human cells using engineered dCas9-methyltransferases. Sci. Rep. 2017;7:6732.
118. Morita S. et al. Targeted DNA demethylation in vivo using dCas9-peptide repeat and scFv-TET1 catalytic domain fusions. Nat. Biotechnol. 2016;34:1060–1065.
119. Xu X et al. A CRISPR-based approach for targeted DNA demethylation. Cell Discov. 2016;2:16009.
120. Shalem O et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. Science 2014;343:84–87.
121. Wang T, Wei JJ, Sabatini DM, Lander ES. Genetic screens in human cells using the CRISPR-Cas9 system. Science. 2014;343:80–84.
122. Koike Yusa H, Li Y, Tan EP, Velasco Herrera Mdel C, Yusa, K. Genome-wide recessive genetic screening in mammalian cells with a lentiviral CRISPR-guide RNA library. Nat. Biotechnol. 2014;32:267–273.
123. Doench JG. Am I ready for CRISPR? A user's guide to genetic screens. Nat. Rev. Genet. 2018;19:67–80.
124. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, et al. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013;339:819–823.
125. Guan Y, Ma Y, Li Q, Sun Z, Ma L, Wu L, et al. CRISPR/Cas9 mediated somatic correction of a novel coagulator factor IX gene mutation ameliorates hemophilia in mouse. EMBO Mol. Med. 2016;8:77-488.
126. Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, et al: Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-C as system. Cell. 2015;163:759-771.
138. Brenner, S. Life sentences: Detective Rummage investigates. Genome. Biol. 2002;9:1013.1–1013.2
139. Hsu, P. D., Lander, E. S. & Zhang, F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014;157:1262–1278.
140. Pulecio J, Verma N, Mejia Ramirez E, Huangfu D, Raya A. CRISPR/Cas9-based engineering of the epigenome. Cell Stem Cell. 2017;21:431–447
141. Barrangou, R. &Horvath, P. A decade of discovery: CRISPR functions and applications. Nat. Microbiol. 2017;2:17092
142. Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. Nat. Biotechnol. 2014;32:347–355
143. Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014;346:1258096.
144. Fellmann C, Gowen BG, Lin PC, Doudna JA, Corn JE. Cornerstones of CRISPR-Cas in drug discovery and therapy. Nat. Rev. Drug. Discov. 2017;16:89-100
145. Wang H, La Russa M, Qi LS. CRISPR/Cas9 in genome editing and beyond. Annu. Rev. Biochem. 2016;85:227–264.
146. Dunbar CE et al. Gene therapy comes of age. Science. 2018;359. DOI: https://doi.org/10.1126/science.aan4672
147. Baltimore D et al. Biotechnology. A prudent path forward for genomic engineering and germline gene modification. Science. 2015;348:36-38
148. Gantz VM, Bier E. Genome editing. The mutagenic chain reaction: a method for converting heterozygous to homozygous mutations. Science. 2015;348:442–444.
149. Gantz VM et al. Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito Anopheles stephensi. Proc. Natl Acad. Sci. USA. 2015;112:E6736–E6743.
150. Hammond A. et al. A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae. Nat. Biotechnol. 2016;34:78–83.
151. Oye KA et al. Biotechnology. Regulating gene drives. Science. 2014;45:626–628.
152. Esvelt KM, Smidler AL, Catteruccia F, Church GM. Concerning RNA-guided gene drives for the alteration of wild populations. Elife. 2014;3. DOI: https://doi.org/10.7554/eLife.03401
153. Charlesworth CT et al. Identification of pre-existing adaptive immunity to Cas9 proteins in humans. BioArxiv; 2018. DOI: https://doi.org/10.1101/243345
154. Moreno AM. et al. Exploring protein orthogonality in immune space: a case study with AAV and Cas9 orthologs; 2018. Available:https://www.biorxiv.org/content/early/2018/01/10/245985

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