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Review

CRISPR/Cas system: A game changing genome editing technology, to treat human genetic diseases

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ABSTRACT

Genes, are the functional units of heredity that used as an instructors to make proteins either to become the functional or structural part of the cell. Hence, the proteins get more attention because most of the life functions depends on it. Any mutation or alteration in genome sequences results in complete loss of function or formation of abnormal protein which leads to hereditary disorder. Gene therapy on the other hand, used as a remedy, a process that make correction in the gene which is responsible for genomic disorders. The treatment of disease state depends on the understanding of their genetic basis. While, numerous molecular genome editing tools have been developed and are being utilized to translate the abstract of gene therapy into reality, but the problem is still a mystery. The genome editing molecular scissors can be applied to dictate the selected genetic products that can have the therapeutic power. Thus, editing the specific sequences depends on the type of strategies being used by a molecule such is HDR or NHEJ. CRISPR/Cas9 editing technology can use in disease model to study the genitival disorders. One side the CRISPR technology seemed to be extremely accurate but on the other side it has some harmful effects i.e. Cas9 proteins sometimes cuts the similar sequences other than the specific targeted and Off-targeting Sequences etc. Urgent attention and improvement are needed for various implication of CRISPR/Cas9 technology, including the delivery, precision and control over the mention system. This review presents the current scenario of genome editing in vivo and its implications for the future of human genetic disease treatment as well as genome throughput potency.

1. Introduction

Genes, the specific sequences of Genome and functional unit of heredity that encode instructions to make protein either for structural or functional part of the cell. The proteins are the central part in body functions. Any defect in gene which encode a specific protein are unable to carryout normal body function leads to genetic disorders. So aptly, James Watson said, “we used to think that our fate was in our stars, but now we know, in large measures, our fate is in our genes”.

Gene therapy, defined as the practice using genes as medicines for the precision of defective genes responsible for a genetic disorder (Miller, 1992; Verma and Weitzman, 2005). Though having a prospective future, it is still in experimental stages and many researches remain to be done before in the applications of gene therapy becomes a reality. Its possible applications are single gene recessive disorders and acquired genetic disorders (Kim et al., 2017a).

Various molecular tools have been developed and are being utilized to translate the abstract of gene therapy into reality (Fig. 1). Essentially, gene therapy is based on the concept of genome editing, which in turn, is based on creating the DSBs (DNA double strand breaks) in backbone

Abbreviations: CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; DSBs, DNA double strand breaks; NHEJ, non-homologous end-joining; HDR, homology directed repair; ZFNs, Zinc Finger Nucleases; TALENs, Transcription Activator-like Effector Nucleases; gRNA, guide RNA; DM, Duchenne Muscular Dystrophy

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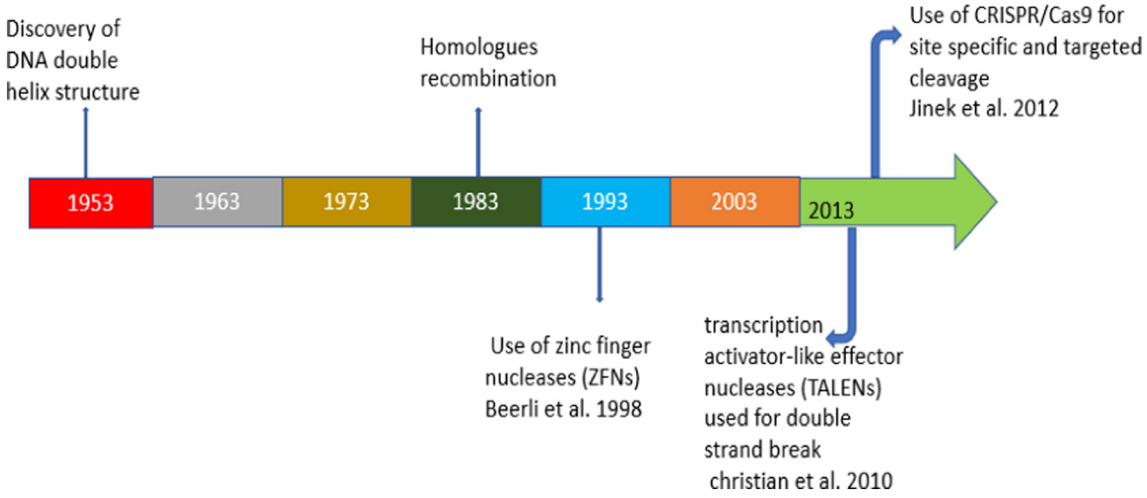

Fig. 1. History of Genome editing technology research.

at specific site of a gene (Hsu et al., 2014). The DSBs, however, needs to be repaired for normal gene function. The cell system employs two competing mechanisms to repair the DSBs: via either non-homologous end-joining (NHEJ) mechanism or homology directed repair (HDR) mechanism. Non- homologues end joining (NHEJ) is an error correcting method and gives rise either deletions or incretions leading to changes in protein coding sequences. HDR, on the other hand, as a type of homologues recombination with the incorporation of a donor DNA sequences to introduce the desired and precise incretions or mutations in the target locus (Lin et al., 2014; Irion et al., 2014; Baena-Lopez et al., 2013; Maruyama et al., 2015). The genome editing on HDR-based, led to multiples research development customs which can use to precisely make double-strand break in specific target sequences manner. Consequently, about four major genome editing molecules have been presented which having the potency to induce the DSBs in vivo or in vitro such is ZFNs (Zinc Finger Nucleases), TALENs (Transcription Activator-like Effector Nucleases), Mega Nucleases and the most recent and precise technology CRISPR/Cas9 system.

Ishino et al. (1987) discovered scarce DNA repeats which have unknown function in the genome *Escherichia coli*. Later, Mojica et al. (2000) identified similar repeats in other microbes and termed them as Clustered Regularly Interspaced Short Palindromic Repeats or CRISPR (Lander, 2016; Mojica et al., 2000; Jansen et al., 2002). Later these sequences (CRISPR) was proved about a mechanism of adaptive immunity evolved by bacteria to destroy the DNA of viral pathogens by cutting with their Cas nucleases (Jinek et al., 2012; Barrangou et al., 2007).

2. Biology of CRISPR system

Biology of CRISPR protein (Cas nucleases) are pathogen-specific and requires guide RNA (gRNA) sequence. The gRNA complimentarily fixes to the specific or selected target portion of the DNA and direct the Cas nuclease for causing DSBs on that targeted DNA sequence. The unique property of the Cas nucleases is the recruitment of gRNA at the desired targeted site has made the Cas nucleases as high-fidelity genome editing candidates, at any target location in the genomic DNA in vivo. In all Cas nucleases, however, Cas9 nuclease of the *E. coli* has the most studied owing its sequences specificity and flexibility. CRISPR/Cas9 module is produced with its two crucial parts the gRNA and Cas nucleases. The specificity of targeted site of the DNA determined through complimentary base pairing on gRNA, while co-localizing the Cas9 to the same specific targeted DNA sequence. This complex module cuts the genomic DNA sequences by binding onto complimentary base pairing followed by localizing of Cas9 at specific site (Fig. 2).

3. Application of CRISPR system

CRISPR/Cas9 gRNA genome editing system has been explored for basic biological sciences in experimental studies including invertebrate and mammalian system. In an alternate study the CRISPR technology has been used with its custom gRNA (guide RNA) in vivo in human by targeting the AAVS1 locus, they obtained 10 to 25% in 293T cells, 8 to 13% in K562 cells and 2 to 4% in pluripotent stem cells. They showed that whole process of CRISPR system depends on its components like simultaneous incorporations of multiples guide RNA (gRNAs), which increase the potency of editing target sites. Results were established that gRNA genome editing is the simplest and vigorous for the multiplex editing of human genome (Cox et al., 2017).

Using the CRISPR associated Cas9 (endonuclease) also have the potency to target editing genome in *Caenorhabditis elegans* germline using single gRNA with U6 small nuclear promoter of RNA. Results showed that targeted, heritable genetic alterations with good results could be achieved in *C. elegans*. Mention technology can also give an easy way as well as effective approach for generating loss of function in it (Gaudelli et al., 2017).

CRISPR/Cas9 nucleases can also persuade DNA DBs (double strand breaks) the specific targeted sites and then it could be repaired by using the Homologues recombination. The phenomena of using this methodology to produce the targeted mutations in *C. elegans* at lower cost and little time, is an important strategy which is already presented in tractable microorganism (Brinkman et al., 2014).

CRISPR/Cas9, application only needs the designing of specific sequence of gRNA, which are complementary to any target region to recruit Cas9 nucleases to the targeted region. Thus, Homologues recombination (HR) is the determined momentous breakthrough by establishing gene target through homologous (HR) (Carrington et al., 2015). Using animal as a model, now it's possible to exactly operate at all gene, knockout and knock-in through mediation of homologues recombination, but HR-mediated targeting is very slow and laborious expertise. To resolved this circumstance, a series of nucleuses has been developed such is ZFNs and TALENs (Dabrowska et al., 2018; Anker and Schaaf, 2000), as compare to these mention genome editing technologies, Although CRISPR/Cas9 has an important application in science, but there are several properties that effect its specificity and efficiency such as target site selection, short gRNA design, off target effects, delivery methods and homologues directed repair (Schaefer et al., 2017).

On Application basis, either one or more different vectors are used to express these molecules by making vector construction of the Cas9 and gRNA, then transformation into the targeted cells. Custom-based

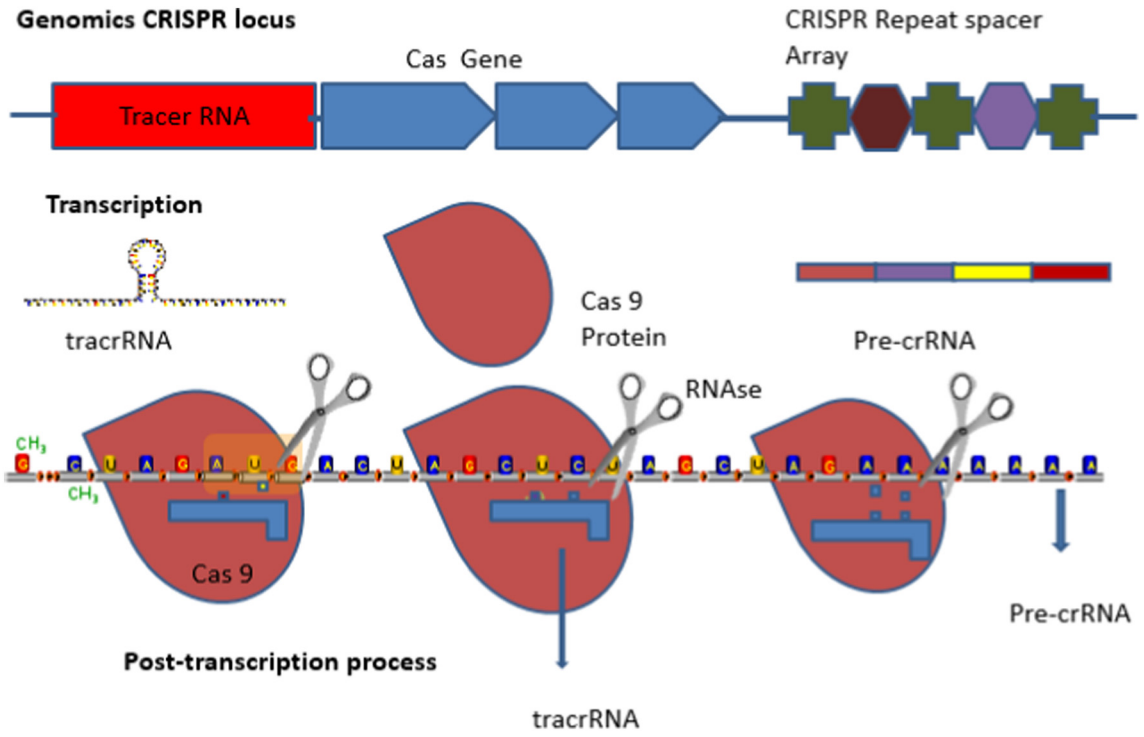

Fig. 2. General biology of CRISPR Cas genome editing system, Transcription occur in the genomics locus and make transcribed into Cas9 enzyme, tracrRNA, pre-crRNA and Cas 9 enzyme and, these all are further combine and form a pre-crRNA and tracrRNA duplex led the recruited the Cas9 to generate double strand break at target sites. In conclusion, the HDRs and NHEJ repaired the DSBs.

optimized nuclease activity and binding properties of Cas9 are being constantly sought out using DNA recombinant technology to identify Cas9 orthologous having different characteristics, such as selective binding and changes in nuclease act with different properties, such as changes in activity with many other applications (Table 1).

In some cases, type of Cas9 mutants such is mutant Cas9D10A, nickase was established that have ability to make precisely selected ssDNA cuts at the specific selected target site of DNA sequences (Ran et al., 2013). The discovery of CRISPR and its associated nuclease, recently gave rise an idea for the researchers can able to precisely correct the *CFTR* gene using the intestinal stem cell organoids of CF patients (Komor et al., 2016).

In the human *F9* gene, type B hemophilia contain a unique mutation (Y371D), which was identified using CRISPR/Cas9 technology in a genetically modified model, it was also confirmed that it cause severe hemophilia as compared to the previous identified mutation (Y371S). Therapeutic method establishment for the mentioned mutation, adenoviral was used to carry and deliver the Cas9 to the targeted site in adult mice. Thus, using this phenomena the infected hemophilic mice received the naked DNA and more than 0.56% corrections were established in the hepatocytes (Nishida et al., 2016).

Furthermore, the inherited X-linked diseases like Duchenne Muscular Dystrophy (DM) caused due to mutation in a gene, which transcribed and make dystrophin protein responsible for muscles fiber integrity. It was characterized by progressive muscles weakness and shortlist life Spain, so there is no effective treatment available for the said disorder. CRISPR/Cas9 genome editing on the other hand, showed 2 to 100% successful correction was made in *dmd* gene, by using the *mdx* mice germline and then monitored the muscles structure and function (Kuscu et al., 2017). CRISPR Cas9 technology was also used for the editing of human blood progenitor 13 nucleotides sequences, which are present in the HBGI and HBG2 genes for the therapeutic purposes of β-hemoglobinopathies. Thus, for reviewing a naturally occurring HPFH-linked mutation by using CRISPR system therapy, the results presented the edited progenitors produced RBCs in great amount of HbF which leads to inhibition of pathological hypoxia induced RBC found in CSD (Kim et al., 2017b).

CRISPR Cas9, can give the opportunity to use for genome editing in broad range as well as for the treatment of pre-clinical trials of monogenic disease. That are currently difficult or even incurable. Several novel type of single-polypeptide CRISPR-Associated systems has been discovered i.e. Cas12a/Cpf1 and Cas13a/C2c2. These additional systems have novel structure and function features providing great opportunities for genome editing (Bak et al., 2018; Wu et al., 2018).

4. CRISPR using as a base editor

The cytidine deaminase base editors (Bes) fused to CRISPR-Cas9 enable to convert the cytidine to uridine by leading the single-base-pair substitutions in eukaryotic cell. By delivering BE with mRNA or ribonucleoproteins by targeting the Tyr or Dmd via microinjection or electrophoresis in mouse zygote. The mice showed an efficiency of 55–57% as well as allelic frequencies of to 100%. It is an efficient method to generate targeted mutation in point mutations (Kim et al., 2017a).

In an another study engineered fusion of CRISPR/Cas9 and a cytidine deaminase enzyme with gRNA without inducing dsDNA breaks and mediates the direct conversion of cytidine to uridine by effecting C → T or G → A substitution. The BE convert cytidine at about approximately five nucleotides and correct a variety of point mutation relevant to human disease. In the transformed human and murine cell lines, second and third generation BE with fuse uracil glycosylase inhibitor which were used as a Cas9 nickase manipulate the cellular DNA repair responses in desired base-editing outcomes resulting in permanent correction of 15–75% (Komor et al., 2016).

Induced DNA damage through CRISPR-Cas9, may have some deleterious effects at the region which exist high-copy-number. Using CRISPR base editors to knockout genes by change of single nucleotide to generate stop codons. This method is more efficient and less deleterious as compared to wild-type-Cas9 for gene knockout studies. Stop

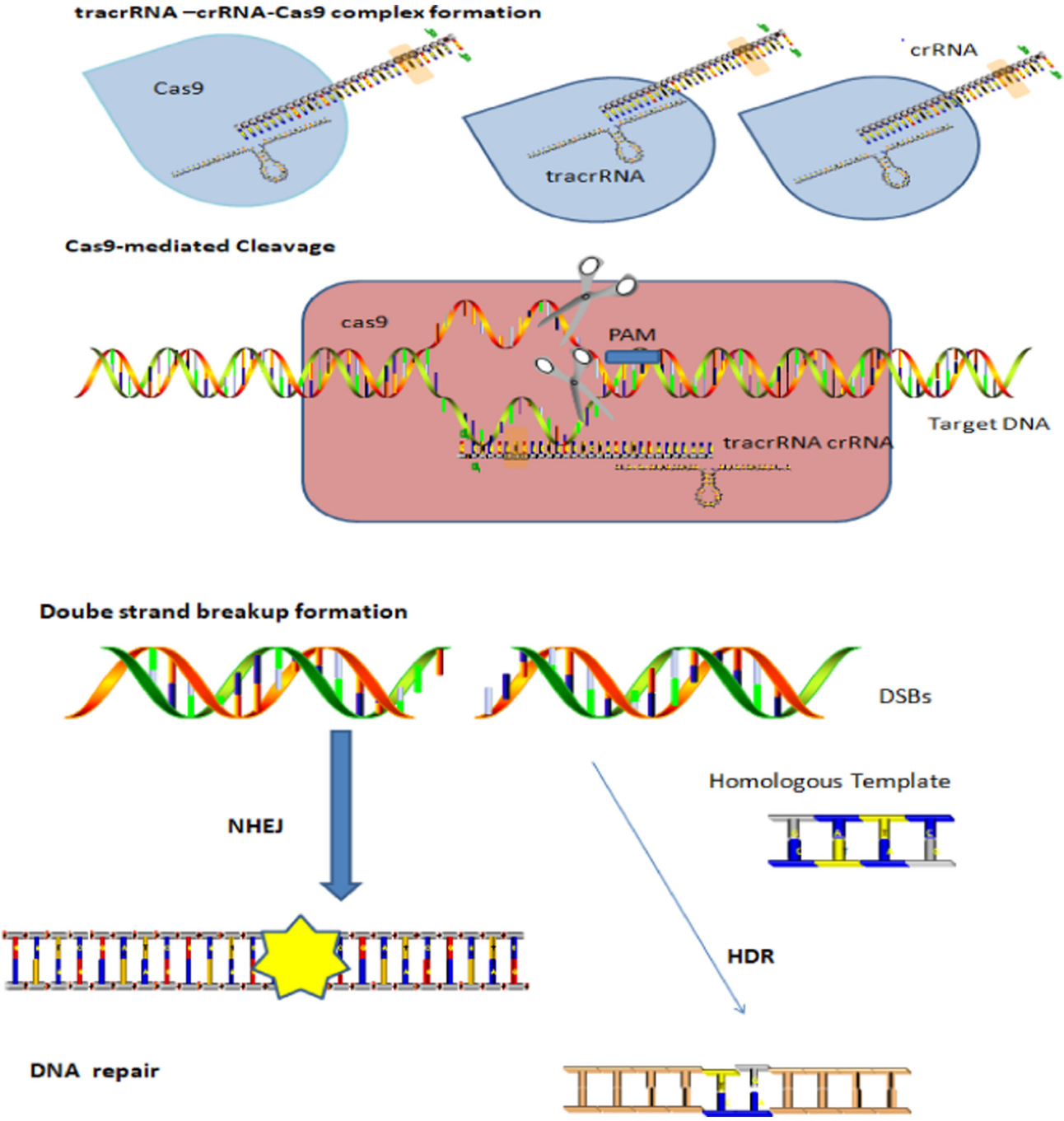

Fig. 2. (*continued*)

codons can be generated in ~17,000 human genes. CRISPR stop mediated targeted screening presented good efficiency as compared to WT Cas9, which is an essential and suitable approach for genome wide function examination (Kuscu et al., 2017).

Genome editing of nucleic acid gave great opportunity for specific targeted genetic diseases at RNA level and lead to correction of disease relevant sequences protein. The type IV CRISPR-Cas systems and its associated RNA-guided ribonucleases Cas13 which having the capability of knockdown and demonstrated RNA editing by using catalytically inactive Cas13 (dCas13) to direct adenosine-to-inosine deaminase activity by ADAR2 (adenosine deaminase acting on RNA type 2) to transcribed in mammalian cells. Engineering this system to create a high-specificity to edit full-length transcript containing pathogenic mutation, this system is referred is REAIR (RNA Editing for Programmable A to I Replacement). Repair presents a good result for in RNA editing platform in research, Biotechnology and therapeutics proposes (Cox et al., 2017).

In human half of the pathogenic point mutation is due to spontaneous deamination of cytosine, which is major source of transition from C-T to T-A and it is do difficult to convert it again to correct sequences to treat the genetically diseases. Adenine deamination results into inosine which then treated with guanine by using the polymerases but still there is no information about an enzyme which deaminized the adenine in genome. So overcome the problems adenine base editors (ABEs) were used by mediating conversions of A-T to G-C by evolving of transfer adenosine deaminase that operation on DNA when fused to catalytically impaired CRISPR Cas9 mutants. Now the researchers have make possible that the seventh generations ABEs proteins engineering

Table 1
CRISPR/Cas genome editing base studies for human genetic diseases.

S. no	Genetic diseases	Mutation	CRISPR/Cas9 system	Function test	References
1	Chronic Granulomatous Disease	Mutation in CYBB gene	CRISPR/Cas9D10 nickase through HR	In vitro differentiated macrophages	(Cai et al., 2016)
2	Barth Syndrome	Deletion or Mutation of 1 bp	CRISPR/Cas9 and piggyBac for HR	In vitro differentiated Cardiomyocyte muscles	(Wang et al., 2014)
3	β-Thalassemia	HBB gene Mutation	CRISPR/Cas9 and piggyBac for HR	In vitro homoeotic differentiation gene expression	(Xie et al., 2014)
4	β-Thalassemia	A/G and TCTT deletion in the HBB gene	CRISPR/Cas9 and piggyBac for HR	In vitro homoeotic differentiation gene expression	(Xie et al., 2014)
5	Hemophilia A	Gene Inversion in Intron 1 to 22	Cas9+Grna in plasmid were delivered	In vivo in hemophilia mouse transplantation in endothelial cell	(Park et al., 2015)
6	Cataract	*Crygc* gene	Injection of sgRNA, Cas9 mRNA and ssODN in template form	Gene repair strategy in zygote through HR	(Wu et al., 2013)
7	Urea Cycle Disorder	*OTC*	2 AVV for the expression of gRNA and Cas9 in donor DNA	In vivo target of gene By CRISPR system	(Yang et al., 2016)
8	Cholesterol Regulation	*PcsK9*	*S. pyogenes* Cas9+AVV	In vivo test in mouse liver	(Ran et al., 2015)

can have completely converted the targeted A-T to G-C about 50% efficiency in human cells. Now this discovery makes it possible which have used to correct the diseases and diseases-suppressing mutations in human cells with addition ABEs enables the direct, programmable introductions of all four transitions mutations without double-breaks of DNA strands (Gaudelli et al., 2017).

The efficiency to induced mutation for genome editing mainly depend upon the targeted sequences. So for this task an accurate, quick and simple method assay is necessary for quantification of induced mutation. Luckily, TIDE method have overcome these problems. It is a method which only need a pair of PCR reactions in which two capillary sequences run, these sequences trace and analyzed by special developed decomposition algorithm that identifies the induced mutations in the edited site also their frequency in cell. This method is quick, cost effective and provide details information's then other available enzyme-based assay (Brinkman et al., 2014).

CRISPR Cas9, a broad range genome editing and engineering technology that only depends on single guide RNA (sgRNA) and Cas9 enzymes. Fast, simple and cost effective method for generation of targeted mutagenesis in culture cells. i.e. Zebrafish, mice and different model organism, but pre-screening of sgRNA for targeted efficiency also for successful mutagenesis and minimizing waste animal's models in experiments. For this purposes a quick, cost effective and easy Fluorescent polymerases reaction (PCR)-based method has been developed known is CRISPR Somatic Tissue Activity Test (CRISPR-STAT). it used for targeted specific efficiency of sgRNA. It is validated in 28 sgRNAs and have greater level of germline efficiency in zebrafish. It can also be applied for other models organisms to overcome the mutations rate and find the targeted efficiency of the sgRNAs in human too (Carrington et al., 2015).

5. Harmful effects of CRISPR Cas9 system

The discovery of CRISPR Cas9 systems and its progress in the field of treating human disease especially the Human genetically disease make it possible to eliminate many hereditary diseases in the near Future. The technology has given a toll to the scientist to cut replaced or delete the specific targeted mutation in the genome by using the Cas9 proteins and gRNA for therapeutic proposes. In one side the CRISPR technology seemed to be extremely accurate but on the other side it has some harmful effects i.e. Cas9 proteins sometimes cuts the similar sequences other than the specific targeted and Off-targeting Sequences etc.

A recent study published in nature in which CRISPR Cas9 were used for the restoration of sight in blind *rd1* mice by correcting the mutation of *Pde6b* gene. However, a secondary mutation was identified in the regions which were not targeted by the guide RNA (sgRNA). It was more than the expecting targeted loci in mice and it was identified is Off-Targeted effects of the CRISPR Cas9 gRNA and Cas9 protein which have targeted the unwanted similar sequences in *rd1* mice genome (Schaefer et al., 2017). It was also identified that the off-targeted effects of the RNA guided nucleases were more than 50% induced mutation at sites other than the intended on target site (Zhang et al., 2015), it is a major concern especially for the therapeutic and clinical trials of CRISPR Cas9 system. So to overcome this problems basic mechanisms such is methods for detecting off-targeted effects, underlying off-targeted cutting and strategies for minimizing off-targeted effects as well as human friendly therapy is still needed to facilitate the basic and clinical application of this technology.

6. Conclusion

Marvelous development has been made to address the challenges in conventional genome editing therapy, through creating advanced technologies for the precise and selected target sites modification of human genome. These technologies build a bridge towards success for researcher and removed the obstacles that have overcome the field of

genome editing for so many years. The 20th and 21th centuries blessed the field of genome research with so many gifts, the discovery of CRISPR/Cas9 technology is one of them. Using this technology, we are now able by building a powerful genetic animal model organism for the understating of human genetic diseases. Even in the begging, this technology also enabled genetic research and have strengthen our grip for molecular studies and successful correction in diverse genetic disorders.

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