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A Systematic Review on the Comparison of Molecular Gene Editing Tools

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Abstract:- The advancement and latest technologies used for genome editing has opened the door of targeting and altering the genomic sequences, depending upon the bacterial nucleases, in almost all eukaryotic cells. Genomic editing has helped the scientists in understanding the contribution of genetics in studying pathological process of many diseases by creating accurate animal and cellular models. This technique has shown extraordinary potential and provided latest approaches from basic research to advance biomedical and biotechnological research. The idea of gene editing technology has reached the clinical practice phase after the discovery of programable gene editors like Zinc Finger Nucleases (ZFN), Transcription Activator Like Effector Nucleases (TALENs) and Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)–Cas-associated nuclease. This review will discuss all the three strategies (ZFN, TALENS, CRISPR/case system) used for genome editing and their application against the diseases as well as their mode of action and delivery methods.

Keywords:- *Genome editing; Zinc Finger Nucleases (ZFN); Transcription Activator Like Effector Nucleases (TALENs); styling; Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)–Cas9.*

I. INTRODUCTION

Molecular biology has paved the path for the clinical healthcare system. One of the many advantages is the method of altering the codes of DNA which is also known as genome editing. It involves the editing and modification in DNA from the preselected loci that uses artificially created endonucleases which have the capability to alter abnormal cell functioning [1]. Genome editing uses two domains in its functioning that can be synthesized by any known sequence of DNA i.e., Cleavage Domain and the DNA binding domain. Now, genome editing is the everyday routine in genetics laboratories [2]. More than two decades ago the idea of genome editing arose, when during homologous recombination scientist realized the need of inactivation, addition and alteration in DNA was needed [3].

The world of biotechnology is witnessing a continuous outburst of many molecular techniques after the discovery of "post DNA helical structure". These tools that are now being used in many laboratories and research purposes and has provided the scientists with the chance to micro-edit genomes and messenger RNA. In order to improve the yield and specificity of an edited product, platforms for these techniques are being updated and improving everyday by latest discoveries and technology miniaturization.

Until the discovery of genome editing in the mid twentieth century, scientists like "Mendel, Morgan, Avery, et al" relied on the discovery of spontaneous mutations only. Then, in the next few years, Muller and Auebarch presented the idea that by using chemical radiation and laboratory treatments the rate of mutagenesis could be enhanced [4]. The insertion of transposons played a key role for genome editing, but these techniques were able to produce alteration only in the random sites of the genome. In the late 20th century, first genome changes were introduced in mice and yeast, the technique used for gene editing was homologous recombination but it was inefficient for mouse. Owing to the absence of culturable embryonic stem cells in mammals (other than mice) and low frequency, the technique of gene targeting could not be used for other species. But, with the latest advancement in the molecular techniques and discovery of nucleases for genome editing tools, now the manipulation of gene could be done for any of the species and cell. The latest researches in the system have confirmed the notion of Nobel Laureate Sydney Brenners, "Progress in science depends on new techniques, new discoveries and new ideas, probably in that order." (http://www.azquotes.com/author/24376-Sydney_Brenner)

For the first time, nuclease free gene targeting was used against mouse in "1986". Later with development and advancement in the field of genetics and molecular biology, first nuclease system ZFN was created in "1996". Before the discovery of ZFN, many researches were made by the scientists for TAL effectors and CRISPR [5]. The core concept of the idea was that site specific DNA double stranded breaks (DSBs) could be created by genetically engineering the nucleases. This idea of gene editing has opened doors for using this as research tool from basic level to biomedical science and applied biotechnology. Natural phenomenon of repairing the DNA breaks is masked by this new and advanced technique of genome editing using different nucleases. In the start two methods were used for the genome editing that made use of endonuclease and DNA binding proteins i.e., Zinc Finger Nuclease (ZFN) and Transcription Activator Like (TAL) effectors respectively.

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ZFNs and TALENs were made by the fusion of Zinc fingers and TAL effectors to the DNA cleavage domain "Fok1". As Fok1 is a dimer, so for this purpose the molecular designing of ZFNs and TALENs must be such that it brings the two domains of Fok1 together for the catalytic activities [6]. Based on the bacterial immune system one of the most common and advanced platforms used as nucleases for genome editing is "Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9" nuclease system. The mechanism of action of Cas-9 depends on the 100 nucleotide "Single Guide RNA (sgRNA)". Cas-9 is activated and directed to the site of action where it targets the DNA and cleave chromosomal specific site by sgRNA. Cas-9 has gained more popularity and preference among all other nucleases platforms because of its specificity of action, speed and low price as compared to other nucleases systems used for genome editing.

The ability to make chromosomal sequence of interest from targeted DNA by creating double stranded breaks defines the high efficiency of gene editing. During meiosis, double stranded breaks stimulate the homologous recombination and DSBs formed leads to crossing over of sister chromatids. Highly efficient gene editing tools are used for the repairing of the homologous chromosomes. The other methods used for repairing is the "non-homologous end joining (NHEJ)" that is used for the joining or repairing of the broken ends [7]. Broken ends are joined together with high precision and less often errors occur while restoring the original sequence of DNA. These errors produced during NHEJ results in deletion or insertion of nucleotide bases "Indels". When this genetic error occurs in the sequence of genes, action of gene is inactivated.

To deal with such kinds of errors in genes, we are endowed with three different kinds of gene editing technologies that can create double stranded breaks at nay desired target i.e., Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and CRISPR-Cas [8]. Later one has more preferences in research worldwide as compared to the rest of the two. The fusion of eukaryotic sequence specific transcription factors derived sets of zinc fingers together with the bacterial protein-based DNA cleavage domain give Zinc Finger Nucleases (ZFNs). TALEN has the same technique of forming the hybrid only with difference that it binds the transcription factors of DNA recognition modules that are synthesized from the pathogenic plant bacteria. Last platform of gene editing uses immune system of bacteria to invade DNA and RNA The working methodology of these above-mentioned tools are exhibited in (Figure 1).

ZFNs and Talen's versatility helps in recognizing any sequence of DNA by customizing their DNA binding Domains. Genomic structure and function can be altered by combining the DNA binding domains with effectors these includes numerous repressors, effectors, transposons, histone acyltransferases etc. The alterations in the genome are based largely on the affinity of TALE proteins and Zinc Finger with the DNA binding specificity.

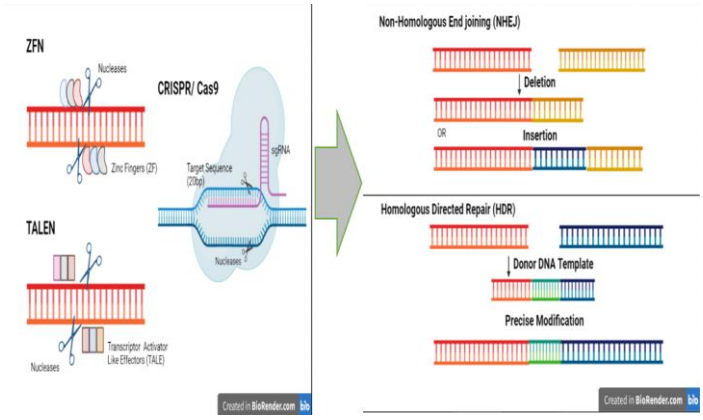

Figure 1: Working methodology of genome editing tools, (Created with BioRender.com)

II. GENE EDITING PLATFORMS:

There are three methods used for the gene editing process.

2.1First Generation Programmable Editor – Zinc Finger Nucleases (ZFNs)

Zinc fingers is the first platform for gene editing techniques. It is actually an artificial endonuclease that uses Zinc Finger proteins that is hybridized with the Foci. Redesigning of Zinc finger can be done with zinc finger with new specificities for new sequences. Zinc finger nucleases (ZFNs) are produced by fusing the zinc finger loaded sequence specific DNA binding domain with the non-specific DNA cleavage Domain. As a part of transcription factor IIIa in the oocyte of *Xenopus*, zinc fingers were discovered for the first time in "1985" with DNA site sequence specific binding properties. By the interaction of sequences of homologous DNA with the domains of zinc fingers, an array of Cys_2His_2 were created for the functional specificity of zinc fingers [9]. A single Cis_2His_2 contains 30 amino acid sequence which further constitute one alpha helix opposing the two antiparallel beta sheets. Among all the transcription factors, one of the most common types of DNA binding motif is the "Cys_2-His_2-ZF" [10]. Zinc fingers by the interaction of their major grove of DNA with the residues of alpha-helix produces base specific contacts.

2.2Mode of Action:

For cleavage of the DNA sequence at specific sites, restriction endonuclease Foci type II is used to form the domain to target the sequence. Two molecules of Zinc Fingers (ZF) are needed to bind the target sequence in proper orientation as ZFN requires dimerization before cleaving the DNA. Double strand breaks (DSBs) start forming once the cleavage is done by the ZFNs, resulting in the modification of repair system of NHEJ and HDR endogenously. Graphical presentation of the mode of action is given in (Figure 2).

There are three methods to determine the specificity and recognition sites of the ZFNs. These includes 1) Quantity of the Fingers, 2) Nuclease domain interaction and 3) Sequence of amino acid for each finger. The structure of Zinc fingers is such that both domains i.e., catalytic domain as well as binding domains both can be optimized which increases the

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chance of high specificity as well as enhanced affinity for the novel model assembly for genome editing. In the past, 9-18 nucleotides were targeted with only 3-6 fingers in the ZFNs. It helped the ZFN dimers to specify a sequence of 18-26 nucleotide bases at each cleavage site in the DNA. Because of the rendering phenomenon of the DNA nucleotide bases, 18 bases are able to render billions of nucleotides bases in the DNA, which consequently led to the targeting of genome editing at specific sequences in the DNA of Human for the first time in the history [11]. The need to improve the accuracy of targeting of ZFN new strategy has developed using the "Selection-based methods". The new discovery has opened the door for two cleavage domains junctions i.e., Finger—Finger and Finger—Foci which has increased the number of configurations for ZFN to about "64-fold" available for the DNA cleavage at any specific site.

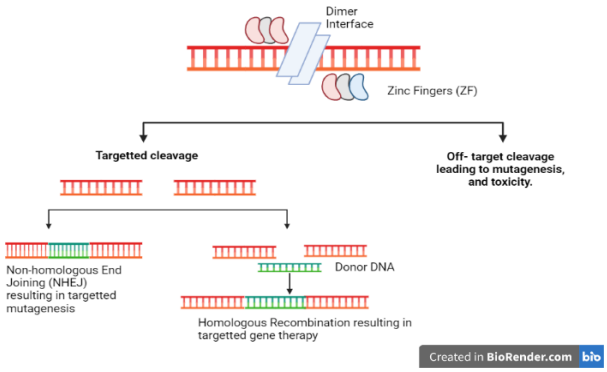

Figure 2: Mode of action of Zinc Finger Nucleases (ZFN) (Created with BioRender.com)

2.3ZFN against diseases:

One of the most interesting and advanced approach of gene editing is the "gene replacement therapy". Gene editing approach using ZFN has proved to be very efficient in modification of hFIX gene in the liver cells of hemophilia B infected mice. Many researches are being carried out for developing a strategy that could replace liver proteins using ZFN. This method will introduce site specific fusion of therapeutic transgenes into the locus of albumin gene in the liver cells.

By using Adeno-associated Viruses (AVV), on the therapeutic level, scientist have achieved the long-term gene expression of hFVIII and hFIX in mouse models infected with hemophilia A and B. As albumin is highly expressed in the liver cells, alterations of less than 1% can be enough to produce relevant therapeutic proteins (effective in curing disorders). Latest researches on ZFN are focused to introduce liver proteins that can be effective against lysosomal diseases i.e., Gaucher, Hunter, Fabry, Hurler etc. many laboratory researches are being carried out to develop gene editing therapies against SCID, Cancer (T cells for immunotherapy), sickle cell anemia [12].

2.4Second Generation Programmable Editor–Transcription Activator Like Effector Nucleases (TALENs)

Transcription Activator Like Effector Nucleases (TALENs) are the second type of gene editors which are better in function and specificity than Zinc Finger Nucleases (ZFNs). TALENs like ZFN also consist of DNA cleavage domain and site-specific DNA binding domain that create Double Stranded Breaks (DSBs). TALENs are also generated by the hybrid of binding DNA to FokI nuclease. The DNA binding domain of Transcription Activator Like Effector Nucleases (TALENs) consist of repeated sequence of highly conserved protein originated from "phytopathogenic *Xanthomonas* bacteria" [13]. The protein helps in modifying the transcription of genes in targeted plant cell. Central region of DNA contains a specific 33- to 35-sequence of amino acid motifs that facilitate the binding of DNA to the TALE. Only with the exception of two sequence of variable amino acids at 12 and 13, rest of the amino acids are similar in structure. For different TALEs, the number and composition of RVDs vary. TALEs play functional role in the plant pathogenesis and has grab scientist's attention because of the ease of engineering, they are used as nucleases and transcription factors. Specificity of binding of DNA is determined by Repeat Variable Di residues (RVDs), in which there are specific nucleotides binding to the specific pattern of the Variable residues. A unique recognition cipher of TALE-DNA is created by one-to-one linking between contiguous nucleotides and the repeated variable Di residues (RVDs) [14].

2.5Mode of action:

Double stranded breaks (DSBs) are created by hybridization of endonuclease FokI to the when DNA binding domain, stimulating DNA recombination to achieve targeted genomic modification. The cleavage domain of FokI must be dimerized for the creating breaks in two strands of the targeted DNA. The structure of TALEN, like Zinc fingers, is designed in pairs in order to bind the loci of target DNA, with 12-30 base pair spacing between the two binding sets [15]. Unlike zinc fingers, linkage between repeated sequence forming long arrays of TALEs that facilitate to target the genomic site, is not redesigned. Following the first genome editor (Zinc fingers proteins), there are many effectors available for genomic modifications, site-specific recombinases, transcriptional activators that support the fusion of TALE repeats. Despite simplicity in design (cipher codes) compare to zinc fingers (Triplet-finger proteins), it has one difficulty in cloning the design of TALE arrays for large scale identical repeats. To solve this problem, many strategies have been developed including "High-Throughput Solid Phase Assembly", "Molecular Cloning", "Golden Gate" and "Connection-independent cloning techniques" to facilitate the fast assembly of TALE arrays [16]. Graphically, mode of action is illustrated in (Figure 3)

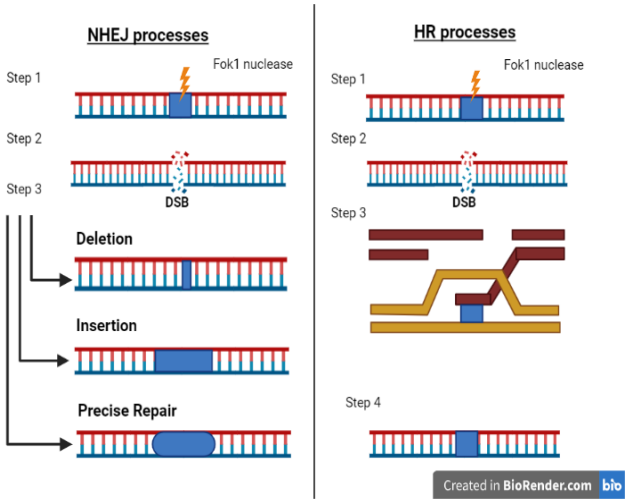

Figure 3: Mode of action of TALEN (Created with BioRender.com)

2.6TALENs against diseases:

TALEN technology has proved to be very efficient in fighting against various disease. Lepr gene was produced in three generation of rats by injecting Leptin receptor (Lepr)into the rat zygotes. The strains illustrated the phenotypical demonstration of obesity with the mutant gene that transferred to the next generation of rat. In "2016" Japanese introduced TALENS into the gamma subunit of interlukin-2 receptor during the pronuclear stage and visually detectable dsDNA mismatches caused the immunodeficiency because of the inactivation of IL2RG [17].

1. **Third Generation programable Editor – Clustered Regularly Interspersed Short Palindromic Repeats (CRISPRs)/ Cas9**

The discovery of third genome editor has revolutionized the field of biotechnology research and treatment against diseases. Clustered Regularly Interspersed Short Palindromic Repeats (CRIPSR) were discovered in bacteria (*E. coli*) in 1987 for the first time and then in many other species of bacteria. The role and function of short repeat sequence remained a mystery for scientist until in 2005 several studies characterized similarities of repeat sequence to Phage DNA. Later further researches showed that these repeats produce RNA-mediated DNA cleavage, and play an important role in inducing adaptive immune response against dangerous foreign DNA [18]. Basically, the CRISPR/Cas system is divided into two systems depending upon the structural variation in the Cas genes i.e., Class 1 CRISPR-Cas systems that contains multiprotein effectors complexes and Class 2 CRISPR-Cas systems that contains one effector protein. Till now, six types of CRISPR/Cas systems and twenty-nine subtypes of Cas-system have been reported [19]. One of the most used and advanced systems of CRISPR is the CRISPR-Cas9 type II system. Cas9 is a more advanced and versatile tool used for gene editing because of the specificity of Cas protein extracted from *Streptococcus pyogenes* (SpCas9) to target specific DNA sequences [20].

III. TYPES OF CRISPR SYSTEM

Depending upon the core of the Cas proteins, CRISPR system could be categorize into classes (1 and 2), types (6) with diverse subtypes (Figure 4).

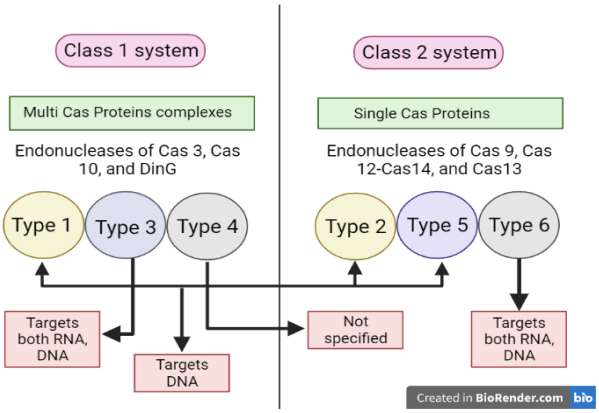

Figure 4: CRISPR/Cas systems (Created with BioRender.com) (Figure 4)

CRISPR/Cas9 type II system:

One of the systems of CRISPR- Cas9 was emerged in 2013 when the world of genome editing was only restricted to two techniques of genome editing i.e., Zinc Fingers (ZF) and Transcription Activator Like Effectors (TALE). There are two components of CRISPR/ Cas9 systems i.e., Cas 9 endonuclease and single stranded guide RNA (sgRNA). The sgRNA is designed in such a pattern to reach the target DNA in a site-specific manner and for the sake of compatibility with the Cas9 proteins used, this step must be followed by the upstream short DNA sequence which is the "Protospacer Adjacent Motif (PAM)" for "NGG" or "NAG" [21]. Double stranded breaks (DSBs) are produced by the cleavage of DNA done by Cas 9 system, stimulated by the binding of sgRNA by Watson Crick Base pairing to the targeted sequence. The DNA-Double Stranded Breaks (DSBs) followed by the DSBs produce by the cleavage, initiates the process of genome editing. Using the CRISPR/CAS9 system, genomic modifications including insertion and deletion (indels) can be made through pathways of Non-Homologous End Joining (NHEJ) or Homologous Directed Recombination (HDR) [22].

In comparison to other gene editing technologies being used, Cas9 system is the most advanced and preferred techniques in the world of gene editing and have many advantages over the other techniques. For instance, reengineering of the enzyme must be done for endonuclease based ZFN and TALEN to fit the target sequences and reengineering of the enzymes must be done separately for each sequence to be edited. But the advantage of Cas9 system over ZFN an TALEN is the versatility of nuclease protein Cas9, to be identical in all the cases and ease of reengineering by changing the guide RNA sequence (sgRNA) to identify new sites to fulfil Watson-Crick base pairing's requirements. As compared to ZFN and TALEN, CRISPR/Cas9 technique require less labor and is less expensive. The other advantage of CRISPR/Cas9 over ZFN and TALEN is the ability of Cas9 to edit at multiple loci simultaneously, make this technique

more scalable and easier. CRISPR is able of performing the gene insertion "knocking novel genes", deletion "knockout existing genes" and DNA or RNA base editing. Among all of the other techniques being used, base editing is one of the newer and authentic methods of generating accurate point mutations in DNA and RNA by using enzymes and other components [23].

3.1 Mode of action:

The mechanism of action of CRISPR is different from all other techniques of genome editing. In this method the DNA is inserted into loci of prokaryotic genome. Primary transcript is made by transcription of CRISPR loci, this is further processed to give the CRISPR RNA or the crRNA via trans-activating RNA (tracrRNA) during the biogenesis process of crRNA. Foreign DNA is cleaved near the PAM region, during interference, by the duplex of Cas 9 endonuclease with the CRISPR-RNA. Mode of action has been illustrated in the (Figure 5).

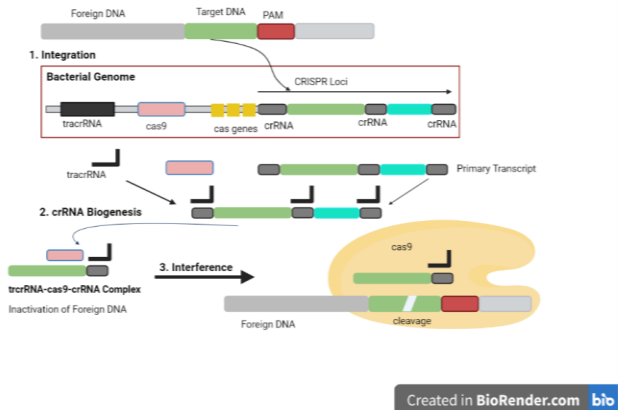

Figure 5: Mode of action of CRISPR/Cas9 system (Created with BioRender.com)

3.2 Limitations of Cas9 system:

DNA editing causes permanent genome changes in the genetic information where this technique is facing many securities and ethical issues. CRISPR/Cas9 system is unable to modify the DNA for some cell type of neuron cells, however, scientists have also worked to produce the editing strategy that only can modify RNA [24]. Downstream proteins are produced by the RNA guided DNA transcription process. CRISPR technology helps in avoiding the unchangeable modification of the genome and in repairing proteins to function efficiently in almost all the cells against the diseases. To treat genetic mutation, another latest approach of combining the stem cell transplantation with the CRISPR/Cas9 can also be used. Research has proven that in order to differentiate retinal precursors, patient-induced pluripotent stem cells (iPSCs) are an excellent cell source that not only are effective in cell replacement therapy but also minimizes the problems faced during immune rejection [25]. The drawback of using the patient-induced iPSCs is that it might produce pathogenic genes and could affect the efficiency of the transplanted cells. The solution to this problem is to fix the mutations that cause disease in patient-induced iPSCs by using CRIPS/Cas9 system, before transplantation [26].

3.3 CRISPR against diseases: Identification of non-coding areas in a genome can be done by CRISPR/Cas9 technology, that result in DNA regulatory elements, illustrating their key role in genomic regulation [27]. CRISPR/Cas system has boosted the generation of new species of animals by genome editing in livestock, to produce gene mutations in animals for biotechnology and medical research. CRISPR has launched "Xenotransplantation" projects or swine models with two main purposes i.e., 1) because it was considered that copies of porcine endogenous retrovirus (PERV) could be activated and, 2) because generation of multitransgenic or better suited pig models were made possible by CRISPR/Cas9 system [28].

3.4 CRISPR-Cas systems in COVID-19 Coronavirus disease 2019 (COVID-19) is one of the worst and the deadliest pandemics ever happened in the history causing respiratory infections, affecting more than 2 million people around the world. For the diagnosis of SARS CoV-2 fast and specific tools have been developed by "CRISPR-Cas systems''. Diagnostic methods for CoV detection by SARS-CoV-2 are much cheaper, specific, easy and more sensitive as compared to RT-qPCR (Reverse Transcription Quantitative Polymerase Chain Reaction) [29].

IV. DELIVERY METHODS FOR GENE EDITING

Delivery methods of the gene editing strategies are as follows:

Zinc Finger Nuclease: The first experiment made for the delivery of ZFN in the *Xenopus* oocyte was done by injecting ZFN synthetic substrate and proteins that were able to break chromatin and stimulate homologous recombination in the cell. A new method of introducing plasmid loaded ZFN and its donors into the human is made possible by techniques of nucleofection, electroporation and available chemical reagents. There are two drawbacks by delivering through0 this method: i) enhanced targeted cleavage of the chromatin and DNA binding, resulting in the accumulation of ZFN in the cell as plasmid continue to express ZFN ii) chances of plasmid to enter into the cell genome. The solution to this problem is transfection of coding of mRNAs together with donor DNA of ZFN into cells. Two types of viruses, "Adeno-associated virus (AVV) and Lentiviruses (LV)" are used for the delivery of donors and ZFNs into the cells of human [30].

Transcription Activator Like Effector Nuclease: Delivery method of TALEN is largely confined to the type of tissues and species. There is a wide range of delivery methods of TALENS into animals, with high transfection efficiency. On the other hand, transfection efficiency of plants is generally low with few options of delivery. Methods used for the delivery of TALEN into the animals includes the methods such as "lipofection", "microinjecting RNA or DNA", "particle-based method", "nucleofection with RNA and DNA" etc. The efficiency of genome editing is affected by the concentration and form differences in the DNA or RNA delivered. According to the scientific research, frequency to cause modification in the genes of the rat embryo during microinjection with RNA is higher than with DNA, observed

modification may also be dose dependent [31]. TALENS are delivered into the body of humans by the action of many adenoviruses, but one of the hurdles for the delivery method is the rearrangement of the TALENs.

Clustered Regularly Interspersed Short Palindromic Repeats (CRISPRs)/ Cas9:

One of the stumbling blocks faced by the CRIPSR/Cas system is the specific and efficient delivery of its CRISPR/Cas components. First method, of delivery is the use of plasmid for the gene editing in which sgRNA and Cas9 proteins are encoded by the plasmid [31] where the sgRNA and Cas9 are assembled in vitro in the same plasmid. Plasmid-based CRISPR/Cas9 is long lasting and preventive against multiple transfections. The key challenge of the system is to introduce the encoded plasmids to the nucleus of the target cells. Second method, includes the direct delivery of sgRNA and Cas9 messenger RNA (mRNA) intracellularly, but the major disadvantage of which is instability of the mRNA [32] that causes modification of genes for short interval and temporary expression of Cas9 mRNA. Third, and the most advanced method includes the direct delivery of sgRNA and Cas9 proteins which is more advantageous in providing greater stability, fast action and limited antigenicity [33].

1) Viral vectors: Adeno-associated-AVV, Lentivirus-LV, Adenovirus-Adv etc.
2) Non-viral vectors: peptide and liposomes nanoparticles.
3) Physical delivery: Hydrodynamic delivery, electroporation and microinjection.

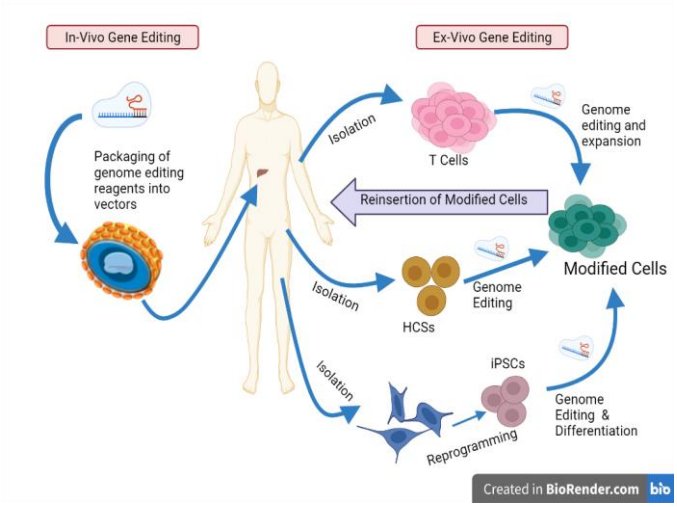

Figure 6: In-Vivo and Ex-Vivo gene therapy delivery methods (Created with BioRender.com).

Comparison:

Comparison of all the gene editing tools that are discussed in the whole article is depicted in the (Table 1)

Table 1: Comparison of different strategies (ZFN, TALEN, CRISPR/Cas9) of genome editing techniques

Properties	ZFN	TALEN	CRISPR/ Cas9
Specificity	Small number of positional mismatches	Small number of positional mismatches	Positional/Consecutive mismatches
Difficulty of engineering	Need protein engineering	Need complex molecules cloning methods	Oligo synthesis and standard cloning methods
Enzymes	FoK1 nuclease	FoK1 nuclease	Cas9 nuclease
Recognition sites	Zinc finger pattern	RVD tandem repeat region of TALE protein	Single strand guide RNA
Delivery methods	Small size of ZFN molecule helps in delivering viral vectors	Large size of complex components cause difficulty	Size of SpCas9 is large and packaging problems such as AVV

V. CONCLUSION

This review demonstrates the tools used for genome editing starting from the most basic tool of editing i.e., Zinc Finger Nuclease (ZFN) to the latest technology for gene editing CRISPR/Cas9 system. Zinc finger nuclease being one of the most basic and traditional technique uses zinc fingers and Fok1 nuclease to create Double Stranded Breaks (DSBs) in the strands of DNA that are further edited either by the non-homologous end joining (NHEJ) or by the Homologous Direct Recombination (HDR). The other strategy discussed in this review, is the Transcription Activator Like Effector Nucleases (TALENs) that works on the same strategy of gene editing as Zinc finger with only few differences i.e., use of TALE instead of Zinc Fingers and uses RVD tandem repeat region of TALE protein as recognition site. Also, the delivery mechanism for ZF and TALE are different and have different road blocks n delivery methods that are still to overcome. Then, in the end, this review focusses on of the most advance method of gene editing known as CRISPR/Cas system. This last technology has zero to no similarity with the other two techniques (ZFN, TALE) and its specificity of action and ease of engineering makes it the most acceptable technique worldwide for gene editing. Unlike ZFN and TALE, CRISPR uses Cas9 nuclease for the modification pattern and tolerates positional and consecutive mismatches. The recognition site used by CRISPR is single strand guide RNA which is different from ZFN and TALN, both.

In the recent few years, CRISPR-Cas system has blossomed as the most-watched and widely accepted tool for accurate gene editing. Still, there is a long road ahead to discover new applications for treatment of incurable diseases because of many challenges in the genome editing strategies. Discovery of CRISPR-Cas9 system has emerged as an expansion in the gene editing tools. To cure many diseases, CRISPR technologies has proven to be very effective and improved over the years to become a novel and accepted gene editing strategy. For specific expression of genes and efficient delivery mechanism on clinical level, CRISPR has to face many challenges.

FUTURE PERSPECTIVES

In the future, information of genetic and epigenetic properties of cancer cell lines combined with pooled CRISPR screening will be able to recognize lethal genome interactions and will help in the discovery of novel drugs in the field of biotechnology. Non coding areas of the genome can also be manipulated by the CRISPR/Cas technology, allowing the exploration of different aspects of gene editing that were poorly understood till now. Development of nucleases has paved a way for genome editing by immense development and advances in the strategies of gene editing tools. Now, single organism can alter the whole local population with the emergence of gene-drive system that engineered mutations in genome can spread in no time. For more latest options to cure human diseases, tumour immunotherapy techniques are also coupled with genome editing technologies. CAR T therapy cell was approved in “2017” as one of the most innovative approaches against tumour immunotherapy. Gene editing technology is paving the grounds in the development of regulation of gene expression, imaging of cell, epigenetic modification, therapeutic development of drug, gene screening and diagnosis.

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