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Molecular Diagnosis of Cancers Helpful Treatments

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Abstract

There is some safety concerns associated with the retroviral gene therapy addressed. Some possible solutions to this problem could be targeted infection, transcriptional targeting, local delivery, co-transduction of retroviral vector with a suicidal gene, specifically targeted retroviral insertion, and SIN vectors. These procedures, with both viral and non-viral systems, can be used in protocols for gene therapy.

Keywords: Cell based therapy; Function of Genes; RNA; Nucleases; Mode of Action; Revolutionary changes;

Introduction

Nevertheless, insertion onco-genesis remains the major concern regarding retroviral gene therapy. A safe and fast means of targeted approach could be the use of foamy viruses. These viruses are harmless to humans and yet have a wide range of hosts. However, for ex-vivo cell-based gene therapy, insertion onco-genesis can be avoided by pre-screening of transduced cells in order to select only those cells which have the transgene only at a desirable site of the chromosome. Molecular biology techniques for the treatment of cancer initially, homologous recombination was used to inactivate the target gene. This was done for characterizing the function of genes. This method was not as productive as it was not efficient in introducing constructs onto the target site. It was very lengthy process, the selection process was laborious and there were many severe mutagenic effects of this method. One of the methods used in cancer therapy is RNA interference. RNA interference involves the use of small non-coding RNA that can bind with other mRNAs and can inhibit their translation into proteins. This can result in the loss of function of genes. In cancer therapy, these RNAi can be used to destroy the function of cancer genes that prevent cancer from spreading [1]. The RNAi method is fast, cheap and it has a high efficiency so it replaced homologous recombination method. Still, it has drawbacks that include incomplete knockdown and temporary prevention of gene function. It also gives off-target effects unpredicted. Cancer recurrence was also seen in some cases. All these problems lead the researchers to explore new methods to alter the gene function. Genome editing is a far better and new technique to treat cancer. Scientists used engineered nucleases that have specific domains that can bind to the target site followed by its cleavage. These nucleases were able to induce double-strand breaks in the target followed by the activation of DNA repair mechanisms. These nucleases were successful in genome editing for curing cancer in different animal models .ZNFs are the first nucleases to be used for gene editing these are found as DNA binding domains in eukaryotes [2]. These are made up of 30 amino acids modules arranged in the form of an array of Cys2-His2 DNA-binding zinc fingers. These modules are used to a nuclease domain of FokI. The modules consist of 3-6 zinc fingers that can identify nucleotide triplets [3]. The FokI nuclease functions only as dimers so a pair of zinc finger nucleases is needed to target any region in the genome. One ZFN will identify the sequence upstream of the genome region to be modified and other will identify downstream sequence. These arrays bind to nearby DNA sequences that are in the opposite strands to induce a double-stranded break in the specific region. The breaks are then repaired by different methods that can cause different changes in the specific region like point mutations, indels or translocations. The ZNFs are custom designed so that they recognize all possible nucleotides and any specific region of DNA. TALENs are similar to zinc finger nucleases, as they also need DNA binding motifs and the same nuclease to edit the genome [4]. The difference lies in the recognition of nucleotides as the TALENs domain identifies only one nucleotide instead of a triplet. The interactions between the TALEN domains and their target sites are stronger as compared to ZNFs. It is easier to design TALENs as compared to designing of ZNFs. Using TALENs for cancer therapy is very effective method. It needs two specifically engineered TALENs that can identify the sequences of DNA in the target gene on the opposite strands. It demonizes the FokI nuclease cleavage domain in the TALENs, cleaving the sequence in the target gene. This causes double-stranded DNA breaks in the targeted gene. The lesion due to the DNA break is repaired by the end-joining DNA repair system. The target gene is altered due to the change in the reading frame [5]. This method can also be used to remove the already present mutations. This gene editing technology can be used to treat the cancer cell lines efficiently as it can target any gene in the genome. Complex cancer genes can be treated by using TALENs. A powerful genome-editing technology known as Clustered regularly interspaced palindromic sequences acronym CRISPR, is now eclipsing all other genome-engineering techniques. This revolutionary technique allows researchers to accomplish targeted manipulation in any gene in the entire genome of any organism in vitro or now even directly in endogenous genome, thus helping to elucidate the functional organization of genome at systems level and identifying casual genetic variations. CRISPR plays a vital role in detection of cancers. If cancer causing gene is known, cancerous cells can be treated with CRISPR-Cas9 system which helps in gene deletion and replacement with a normal gene. The injection process using CRISPR-Cas9 system to cut and introduce gene into liver cells. CRISPR-Cas9 is more effective for single gene mutation cancers and is mostly delivered in vitro in a particular location. In case of metastatic cancers, in vitro delivery becomes difficult. CRISPR primarily constitutes two biological components: Engineered single guide RNA and Cas9. A small guide RNA is used to recognize the complementary sequence specific target flanked by proto-spacer adjacent and it guides endonucleases i.e. Cas9

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to cleave this sequence. Different CRISPR-Cas systems have been grouped majorly into three types and sub-types depending on diverse bacterial and archeal repeat sequences, as genes, and their mode of action. Type I and III systems share a common mechanism of processing of pre-crRNAs via specialized Cas endonucleases, and on maturation, each crRNA complex with multi-Cas protein is capable of recognizing and cleaving target sequences which are complementary to crRNA. In contrary to this, Type II system is considered the heart of genome engineering tool because it involves reduced number of Cas enzymes The CRISPR/Cas9 system is preferred over the ZNFs and TALENs because of many advantages [6]. Firstly, the target design process is simpler for CRISPR as it depends upon the Ribo-nucleotide complex formation instead of DNA recognition. It can be designed easily and it is much cheaper than designing nucleases as this does not need different proteins for each target and eliminates laborious cloning steps. This can be used to target any specific sequence in the genome. The CRISPR system is much more efficient than ZFNs and TALENs. The RNA encoding Cas protein can be injected directly for modifying the host genome. It is not so lengthy and laborious process as compared to traditional methods. By using CRISPR, we can introduce multiplexed mutations [7]. Many genes can be mutated simultaneously by injecting with many gRNAs. This process is faster as compared to other methods. It does not introduce sensitivity to DNA methylation so it can be used if the target site is GC rich. Molecular biology has rapidly evolved in the last decade than it has ever before. Different cancer treatment techniques are emerging and succeeding and with the development of ZNFs, TALENs, and CRISPR, scientists are able to target any sequence in the genome, even multiple genes. This will provide immense help in the treatment of diseases like cancer, avoiding the risks caused by the previous methods. It requires non-coding tracrRNA which triggers the processing of pre-crRNA via dsRNA specific Ribonuclease RNase III and cas9 protein. CRISPR loci are predominantly composed of multiple repeated sequences interspaced by variable spacer sequences and Cas genes situated alongside CRISPR locus. First, this CRISPR locus array is transcribed as single RNA, processing of this released pre-RNA into singular shorter units of CRISPR RNAs using host proteins is done. Mature crRNA effectively binds to nucleases i.e. Cas proteins, thus this complex of crRNA-cas9 helps in recognizing and then cleaving the target invading DNA or RNA having complementarity to crRNA. A motif nucleotide called as PAM is located alongside protospacer in CRISPR-Cas systems I & II. PAM, a nucleic acid sequence made up of NGG or NAG trinucleotide for Cas9, flanks at 3' end of the DNA target site, helps Cas9 in its specific cleavage activity and also facilitates Cas9 in distinguishing self-versus non self-bacterial sequences as PAM. SpCas9 is being in use currently for effective genome editing in eukaryotic organisms including humans. PAM located downstream of target is only sequence allowing cas9 target site selection [8]. Cas9 proteins vary in their size and sequence and have common domains as HNH and RuvC endonuclease to cleave two strands of target DNA. Cas9HNH domain is specified for cleavage of strand complementary to guide sequence while Cas9 RuvC domains for non-complementary strand, In addition, Cas9 holds some conserved arginine-rich sites for binding of nucleic acid. Target recognition by this crRNA directs the silencing of foreign sequences by means of case proteins that function in complex with crRNAs. Double-strand breaks in DNA sequence can be repaired by cellular DNA repair mechanisms: the non-homologous end joining or homology-directed repair [9]. The Streptococcus pyogenes-derived CRISPR–Cas9 RNA-guided DNA endonuclease is localized to a specific DNA sequence via a single guide RNA sequence, which base pairs with a specific target sequence that is adjacent to a proto-spacer adjacent motif sequence in the form of NGG or NAG. On induction of double-stranded breaks or nicks at targeted regions, repairing is done by either Non-homologous end joining or Homology directed repair pathway [10].

Conclusion

NHEJ is an error-prone repair mechanism where joining of broken ends takes place, which generally results in heterogeneous indels whereas HDR is a precise repair method in which homologous donor template DNA is being used in repair DNA damage target site

Acknowledgement

None

Conflict of Interest

None

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