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REVIEW ARTICLE

Genome Editing for Morbid Diseases: A Review

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ABSTRACT

Context: Gene editing is a technique involved in making specific changes in the genetic sequence of an organism. Specialized enzymes serve these purposes by cleaving at specific DNA sequences to edit the gene. Using this method an unwanted segment of DNA can be removed, or a desired segment of DNA can be inserted. Thus an organism's genetic constitution is changed or edited. Researchers claim gene editing has the potential to cure diseases and to produce genetically superior human offsprings the so-called designer babies. Despite numerous molecular applications, it is still at the controversial platform for clinical translation. Terror also lies in the creation of bioweapons if this highly sophisticated molecular technology is not wisely used.

Objectives: Gene editing may offer a novel approach for the eradication of morbid diseases.

Materials and methods: Data were obtained and analyzed from electronic database searches of relevant published literatures from Pubmed and Google Scholar.

Conclusion: Utilising gene editing desired specific genetic changes can be made which in the future may help in the complete elimination of deadly diseases.

Keywords: Deoxyribonucleic acid, Ribonucleic acid, Nonhomologous end joining, Homology-directed repair, Zinc finger nuclease), Transcription activator-like effector nucleases, Clustered regularly interspaced short palindromic repeats, Cas9 (CRISPR associated gene).

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INTRODUCTION

Gene editing also called gene engineering is a process by which a portion of desired DNA can be inserted, deleted, modified or replaced in the genome of a living organism. Using a set of core technologies an overall change can be made in the genetic constitution of that particular organism. This is mainly achieved with the help of enzymes called engineered nucleases. The technique is highly sensitive, specific and efficient in executing, necessary modifications done in a particular DNA sequence. With this technique, the desired change both in phenotype and genotype is achieved. This is a powerful tool for controlling gene expression. It has applications in various disciplines of research, treatment of morbid diseases, gene therapy, neurosciences, biotechnology, and agricultural sciences. Emerging trends in gene editing have made researchers implement changes in the human embryos also. Research trials have so far succeeded in correcting deleterious mutations and incorporating protective mutations.[1-3]

MECHANISM OF GENE EDITING

Specialized and programmed nucleases facilitate gene editing by introducing strand breaks. Gene editing works on the principle of double-strand breaks repair mechanism. After creating double-strand breaks in DNA using engineered restriction nucleases the cell will repair the breaks using its natural repair mechanism using two pathways, NHEJ–nonhomologous end joining and HDR – homology-directed repair. HDR involves the generation of a new repair template for ligation whereas in NHEJ ligation occurs without generation of the homologous template. NHEJ factors involved in ligation are Ku 70 and Ku 80, DNA–PKcs, DNA ligases, XRCC4- x-ray cross complementing protein 4, XLF-XRCC4–like factor along with APLF-Aprataxin and PNK–like Factor. All these factors play a major role in ligating the broken strands in NHEJ manner. HDR involved factors are RAD50, MRE 11, Nbs 1, RAD 51, XRCC2, XRCC3, RAD52, RAD54b, and other proteins. NHEJ repair pathways are error-prone and may result in insertions or deletions at the break sites. In a nutshell, the targeted and highly specific nucleases induce double-strand breaks in DNA which are in turn repaired either by nonhomologous end joining method or homologous direct repair mechanism. Thus DNA sequence of interest can be modified either by insertion or deletion.[2-6]

ENGINEERED NUCLEASES

Newly developed restriction enzymes permit targeted modification of any DNA segment in a wide range of

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cells or organism. Genome editing is facilitated by core technologies like:

- Zinc Finger Nucleases
- TALENS
- CRISPR–Cas 9
- Meganucleases

Zinc Finger Nucleases

ZFN is synthetic restriction endonuclease enzymes which specifically bind and cleave segments of DNA producing double-strand breaks at specific. They have both DNA binding domain with zinc binding repeats and cleavage domain (Fok).1 They belong to the most abundant class of transcription factors and typically consists of 3 or 4 zinc finger domains. At the molecular level, zinc finger domain forms a compact structure in the presence of zinc atom and DNA recognition sites are being present in the α helical domain unit interacting with 3 base pairs of DNA. It is a rapid method of gene editing and the mutations made are permanent and heritable. In order to increase its specificity, obligate heterodimer ZFN is developed. Also, protein engineering techniques have been used to increase the cleavage efficiency of ZFN. One major drawback associated with ZFN uses is the generation of off-target mutations.[5-8]

Transcription Activator-like Effector Nucleases (TALENS)

Similar to Zinc finger nucleases TALENS is also a two-component system consisting of a DNA binding domain and a cleavage domain consisting of FokI. In contrary to zinc finger nucleases the DNA binding domain recognizes single base pairs of DNA and has higher gene editing specificity. Other advantages include faster functional nuclease assembly, improved specificity, and reduced toxicity. They can be administered into cells as mRNA or a protein.

Meganucleases

Meganucleases also called as Homing endonucleases are the smallest class of engineered restriction enzymes. In this technique, DNA binding specificity of naturally available homing endonucleases is re-engineered to develop meganucleases. They have limited utility in gene editing applications owing to the difficulty in separating the binding and cleavage domains. Mega TALs is a recently invented restriction enzyme which is a fusion of cleaving homing endonuclease and TALE binding domain. These are highly superior to previous meganucleases and has the potential for enhancing immunotherapies compared to the usual meganucleases.[8-10]

CRISPR–Cas 9 System

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and Cas 9 (CRISPR–associated gene) is an RNA - restriction nuclease enzyme complex. This system is an indigenous adaptive immunity present in bacteria which gives protection against invading viruses and plasmids. It consists of Cas 9 nuclease and single guide RNA containing crRNA (CRISPR RNA) and tracrRNA (transactivating crRNA). Guide RNA is a small piece of RNA sequence which specifically binds to the segment of DNA and also guides the Cas9 enzyme for cleaving. The sequences of guide RNA are complementary to the sequences of target DNA thus enhancing its specificity. Guide RNA is exclusively involved in the recognition of target DNA, and thus this system is considered the most flexible among others. It is faster and cheaper and more accurate.[10-12]

APPLICATIONS AND LIMITATIONS OF GENE EDITING

Gene editing has a wide range of applications in the field of research. It is an efficient tool for prevention as well as treatment of deadly diseases. The focus has been shifted to the formulation of gene therapy using advanced genome engineering. Nonclinical studies and clinical trials have been done using viral vectors. Researches are still ongoing to ascertain whether this therapeutic modality is safe in human beings. Application of gene editing has been done in a variety of monogenic disorders like hemophilia, sickle cell, and cystic fibrosis. Studies are also being done in more complex diseases like AIDS and cardiovascular diseases. Majority of gene editing is done in somatic cells, and so any genetic changes done in germline cells or embryo brings up the ethical concerns and challenges. Embryonic gene editing is illegal in many countries with regards to its safety and ethical norms.[10-14]

CONCLUSION

Gene editing offers manipulation of genetic sequences to achieve a therapeutic effect. Genome editing is the future and boon for the eradication of diseased cells and tissues. It is a breakthrough in the field of molecular biology which opens the possibility of achieving therapeutics against hereditary morbid diseases.

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