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MECHANISMS OF ENDONUCLEASES AND ITS APPLICATION IN HEALTH AND DISEASES: A REVIEW

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ABSTRACT

Genome editing is the process whereby DNA within the genome of organisms is inserted, delete or replaced with the help of nucleases. In this review we focused on the mechanisms of endonucleases and its application in health and diseases. An online search of current and past peer reviewed literatures on Genome editing tools was performed. The zinc finger nucleases(ZFNs), transcription activators like effectors nucleases(TALENs**)**, and clustered regularly interspaced short palindromic repeat(CRISPR) was the major endonucleases discussed. In conclusion the endonucleases are targeted at DNA to exert their action and contribute immensely as therapy for cancer, HIV, Hepatitis B Virus **(**HBV) and correction of defect genes.

KEYWORDS: DNA, ZFNs, TALENs, CRISPR, HBV, HIV.

INTRODUCTION

The genes of living organisms encoded with DNA is the prerequisite for transformation of information from generation to generations in life and editing of the nucleotide genome is through engineered nucleases. Gene editing technology involves the use of biological tools to introduce and modify host DNA through insertion, inactivation or complete removal of faulty gene sequences. Over the years, genetic modification tools have been successfully applied in the alteration of genomic DNA of economically useful plant and animal species (Lombardo *et al*, 2007). However, some of these methods have been ineffective and frequently lacked specificity in their mode of action. Also, this has made genetically modified products inappropriate and unsafe for human consumption and use (Gaj *et al*, 2010). DNA alteration techniques like the use of gene gun, electroporation and microinjection are usually tedious, expensive and faulty in delivering the gene of interest to single cells genome (Wiedenheft *et al*, 2012). The discovery of new and more effective techniques in gene editing technology has increased the possibility of achieving specificity and accuracy in insertion, deletion and modification of DNA sequences of target host genome In recent time, the endonucleases such as; zinc finger nuclease(ZFN), transcription activators like effectors nuclease(TALEN**)**, and clustered regularly interspaced short palindromic repeat(CRISPR) has contributed immensely in germline gene modification, therapy of genetic diseases, correction of gene, species of plants, and many other biological research(Li *et al*.,2014; lin *et al*., 2014; Kim and Kim, 2014;).

Despite their promising prospect, there still exist some limitations in their applicability. However, this review will focused on the molecular mechanism of endonucleases, and it application in health and diseases.

Overview and Mechanisms Action of Endonucleases in Gene Editing

Zinc Finger Nucleases (ZFN)

The **ZFNs** as restriction enzymes with diverse role can be generated by fusion of zinc finger DNA binding domain with a cleavage domain of such DNA. The recognition of nine(9) to eighteen(18) basepairs at the binding domain of the ZFN is due to availability of three(3) or six(6) individuals zinc fingers repeats giving it the ability to facilitate genetic modification at the loci of mammalian genome(Pabo *et al*., 2001). Whereas, the Fokl which serve as the cleavage domain is dimerize

thereby allowing proper cleavage (Bitinaite *et al*., 1998). The facilitation of genetics modification by ZFN is initiated when DNA breaks and become a double stranded which would undergoe repair leading to a desired modification at specific sequence. However, the nonhomologues ending joining (NHEJ) or directed repair pathway is activated when the double stranded breaks is introduced by ZFNs on the loci of DNA resulting in small deletions and insertion thereby causing a disruption of gene (Urnov *et al*., 2010; Lieber, 2010; Carrol, 2008).

Transcription Activator like Effector Nucleases (TALENS)

A chimeric enzymes called **TALENs** which has induce mutation in various orgainism such as; *Caenohabditis elegans*, zebrafish, mouse, rats, xenopus and some species of insects was discovered from a plant pathogen known as Xanthomonas (Aryan *et al*., 2013; Katsuyama *et al*., 2013; Kay and Bonas, 2009). These TALENs consist of DNA binding domain with 10 to 30 repeats tandem allowing recognition of single base pair with the help of amino acid residues(Boch *et al*., 2009; Moscou and Bogdanove,2009). The fokl restriction enzyme served as the non cleavage domain for TALENs. TALENs exhibit it action by cutting the double stranded DNA at a loci, and thereby allowing the already break DNA to undergo repair through Non-homologous end joining or homology-directed pathways leading to disruption, correction or insertion of gene (Takasu *et al*.,2014). However, both ZFNs and TALENs are similar in the generation of Double stranded break(DSB) at a specific genome of target but TALENs tend to be more advantageous than ZFNs in terms of 18-bp Making its specificity less(Guilinger *et al*.,2014). Furthermore, on off target effects, TALENs has fewer mutations than ZFNs when same gene ccr2 is involved but ZFNs upon introduction into cells can generate more toxicity than TALENs(Smith *et al*.,2014).

Clustered Randomly interspaced palindromic Repeats (CRISPR)

The **CRISPR** which can serve as immunity against plamids and viruses is a representative family of DNA repeats constituting 90% archael and 48% bacterial in it genomes(Fineran and Charpenteir, 2012; Bhaya *et al*., 2011; Rousseau *et al*., 2009). At the loci of CRISPR is a spacer and different Cas proteins forming a CRISPR/Cas system and the presence of Cas proteins possess RNase or DNase activity required for most actions of the CRISPR/Cas system(Horvath and Barrangou, 2010; Wiendenheft *et al*.,2009). However, CRISPR/ Cas with the common role of providing immunity can be categorized into three type namely; Type 1,11, and 111 with eleven subtype(Chylinski *et al*., 2014, Makarova *et al*.,2011). The processing of pre-crRNA into crRNA with an induction of cleavage at the targeted site is enhanced by a multifunctional proteins of type 11 CRISPR/Cas system called Cas9. Also, cas1, cas2, cas4, cas6 and cas10 proteins are involved in processing of crRNA (Makarova *et al*., 2011; Karvellis *et al*., 2013). Recognition and target of genetic materials of virus by the CRISPR/Cas systems takes place through acquisition, expression and interference stage. The novel spacer help in the integration of foreign DNA within the locus of the CRISPR after which duplication of DNA occur (Garneau *et al*.,2010). During the expression stage, the Cas proteins such as cas1, cas2, cas4, cas9 and tracrRNA molecule will facilitate the transcription of pre-crRNA at the loci of CRISPR into crRNAs. The already processed crRNAs will move into the Cas complex for antiviral defence which will enable recognition of base pair at the foreign DNA specific target(Deltcheva *et al*.,2011). Finally, at the interference stage, immunity of foreign DNA against pathogenic attack is enhanced when crRNA helps the complex of cas proteins to bind and initiate cleavage at the targeted region of the DNA(Garneau *et al*.,2010). Eventhough, the CRISPR undergoes NHEJ or HEJ repair pathway as ZFNs and TALENs, it Cas9 proteins play a significant role in generating a large vectors for targeting many sites and also help in guiding RNAs which allow simultaneous multiple site targeting in the same cell making it more advantageous than ZFNs and TALENS but become disadvantageous when the Cas9 protein of *S.pyrogen* encoded in cDNA is more larger in size(4.2kb) than ZFNs and TALEN monomer(Gupta and Musunuru, 2014; Mali *et al*.,2013).

Applications of Endonucleases in Gene Editing

Treatment of cancer

Cancer which is caused by alteration in genetic composition (DNA) or mutation of gene can be corrected with the help of CRISPR/Cas9. The cas9 protein will cut the strands of DNA at protospacer adjacent motif(PAM) sequence causing a double stranded break(DSB) leading to disruption and correction of gene. The repair mechanism is initiated by non-homologous end joining with a resultant indel mutation causing disruption in function of the promoter region. That is, an oncogene such as myc in Burkitt´s lymphoma can be disrupt through this process. However, the homologous directed DNA repair will help to repair mutated suppressor gene(White and Khalili, 2016). The oncogenes such as src is inactivated by CRISPR/Cas9 causing disruption in protein motif. In viral oncogenes called Epstein-Barr virus(EBV) which causes Burkitt´s lymphoma and nasopharyngeal carcinoma are targeted by Cas9 leading to low level of viral activity with the potentiality for treatment of cancer(Wang *et al*.,2014; Brown *et al*.,1996; Pagano, 1999). The tumor suppressor which are stopper of cancer cells can also be targeted by CRISPR-Cas9 for correction. For instance, E6 gene which target P53 and E7 gene in complexes with retinoblastoma(pRb) are targeted by CRISPR/ Cas9 in human papillomaviruses ; a causative agent of human cancer(Cervical Carcinoma) with a resultant reactivation of P53 or pRb when cas9, E6- or E7 specific gRNAs is introduce into hela and silta cell lines of cervical carcinoma followed by inactivation of delete and inserted mutation(Kennedy *et al*.,2014; Beaudenon and Huibregtse, 2008).

Hepatitis B Virus Suppression

The prevalence of HBV is so alarming despite the use of nucleotide and interferon as drugs for treatment, and for remedy purpose; this has led to the introduction of ZFNs, TALENs, and CRISPR/Cas9 system for disruption of HBV genome at invivo and invitro **(**Block *et al*.,2015; Lin *et al*.,2014). It is believed that the entering point into the HBV hepatocyte is by binding at the surface receptors leading to the formation of viral template called covalently closed circular DNA(cccDNA) in the nucleus. The core particles(c) which is among the four reading frame of HBV can be transformed to formed more cccDNA at the nucleus. However, the CRISPR-Cas9 will target the entry point with sodium taurocholate co-transporting polypeptide at the receptor by disrupting HBV life cycle. That is, Formation of cccDNA or more recycle cccDNA that can arise from the core particle via translation and reverse transcription is stopped(lin *et al*.,2015). Lin *et al*.,(2014) reported that, combination of HBV-specific Cas9/sgRNA will significantly lead to less production of HBV and HBsAg on co-transfection of Cas9 and plasmid expression of HBV in a Huh7 hepatocyte of derived cellular carcinoma cells. But by extensive finding, Kennedy *et al*.,(2015) used lentiviral transduction of Cas9 and HBV-specific gRNAs to demonstrate how invivo models of chronic HBV infection and denovo infection can inhibits production of HBV DNA and cccDNA accumulation with a discovery that, CRISPR/ Cas9 system help in -1000fold viral HBV DNA and up to -10fold cccDNA suppression level.

Correction of Mutated Sickle Gene(HBB^S)

A sickle cell disease which is caused by a single point mutation at β- globin gene can be corrected with ZFNs and TALENs. The ZFNs target the bone marrow of sufferer of sickle cell disease by correcting the $CD34^+$ cells leading to a wild type production of tetramic hemoglobins(Hoban *et al*.,2015). Whereas, TALENs correct mutated gene(E6V) of patient with derived human induced pluripotent stem cell(hiPSCs) by induction of homology repair along with a donor plasmid constituting a HBB^A gene and *piggy Bac* transposon(Sun and Zhao, 2014; Sun et al., 2012b). That is, upon cleavage of TALENs on specific DSB of HBB^S gene they is stimulation of HR between a genomic fragment and donor plasmid leading to HBB^S being substituted by HBB^A with a simultaneous piggy Bac transposon integration. Sun and Zhao,(2014) reported high specificity and safety for TALENs usage and *piggy Bac* transposon in correcting gene is due to lack of off-target effect and deficit in translocation or alteration of chromosome.

Human Immunodeficiency Virus(HIV)

In HIV treatment, the ZFNs targets CCR5 gene that infect the of $CD4^+$ T cells gene by disrupting such T-Cells with a resistance against Hiv infection**(**Holt *et al*.,2010; Perez *et al*.,2008). Tebas *et al*.,(2014) from an evaluation of patient treated by ZFNs targeting CCR5 gene for a period of 36 week discovered that ZFNs was safer and well tolerated but HIV genomic RNA was undetected in one patients which may be attributed to deletion of 32-bp of CCR5 gene in one allele at the beginning of treatment making ZFNs a temporal approach to remedy HIV infection**(**Ding *et al*.,2013**).** The CRISPR/Cas also target the HIV-1 provirus to cut off the genome of host cells with destruction upon cleavage in HIV-1 repeat terminal regions. Thus, since HIV-1 T cells are usually scattered throughout the body of infected patient it can become challenging for complete elimination of HIV-1 by CRISPR/Cas(Kennedy and Cullen, 2015; Hu *et al*.,2014).

CONCLUSIONS

The endonucleases for genomic editing are targeted at DNA to exert their action with resultant effect of gene correction or deletion via the repair pathway with limited or off target effect. However, this tool has contribute immensely as therapy for cancer, HIV, HBV, correction of defect genes, modification of gene in living organism and other biological process with the CRISPR-Cas being the leading tools. Despite how promising the future is with the applicability of this techniques in health and diseases, there are still some limitations in their use in humans and other biological systems with regards to accurate gene loci targeting and bioethical standards.

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