A Review of Gene Editing/Therapy and CRISPR Technology

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ABSTRACT

This review will discuss the gene editing or manipulation in general, including various methods and tools currently available, including CRISPR. Some encouraging results of clinical applications of gene-therapy will also be discussed. With its diverse and far reaching effect on biomedical field, we will also discuss CRISPR technology beyond its gene-editing functions. We also review the cost for performing a gene-therapy.

Keywords: CRISPR, gene editing

ABSTRAK

Ulasan ini membahas penyuntingan atau manipulasi gen umumnya, berbagai cara dan alat yang kini tersedia, termasuk teknologi CRISPR. Akan disajikan pula hasil-hasil percobaan klinik yang memberi harapan. Mengingat pengaruhnya yang luas di bidang biomedis, akan dibahas pula pemanfaatannya di bidang lain. Akhirnya, akan dibicarakan juga hal-hal terkait biaya pengeditan terapi gen yang sangat tinggi, dengan harapan bisa ditekan di masa yang akan datang dengan teknologi yang lebih canggih. **Khing S. Ong, Zack ST. Lim, Boenjamin Setiawan. Ulasan atas *Gene Editing/Therapy* dan Teknologi CRISPR**

Kata kunci: CRISPR, *gene editing*

INTRODUCTION

The discovery of the double helix structure of molecular DNA 65 years ago by Watson and Crick[1] is the cornerstone of modern genetics. Further advances of technology such as DNA sequencing[2] make the disease gene(s) identification possible. Hence, correction of diseases can be achieved through gene-therapy.

Shortly after the discovery of DNA structure, concept of gene therapy by using a virus vector to carry the healthy version of gene to replace a "mutated" one that causes disease was conceived.[3] However, these earlier attempts worked only sporadically.[3]

In some cases, it even causes disastrous results.[3] Patient treated with virus carrying gene for treating a metabolic disorder, ignited a violent immune reaction and causes the death of the patient.[4] Another instance, children treated for combined immune deficiency-X1 with virus carrying correcting gene, causes leukemia and death.[5] However, researchers were not deterred by these disappointing results, and with the advances and progresses in newer technologies for purifying the virus vector, and the development of CRISPR-Cas9 (cluster regularly interspaced short palindromic repeats-CRISPR associated protein 9) technology had resulted in many successful gene-therapies in the recent years.[6]

TECHNOLOGIES/METHODS OF GENE THERAPY

1. Delivery of the replacement gene(s) :

Basically there are two delivery vectors, viral and non-viral vector. Viral vector will be described under viral transfection method of gene therapy. The non-viral DNA vector commonly used is plasmid (circularized double strand DNA molecule).Using this plasmid as the delivery vehicle, therapeutic gene can be given through a quick injection of a large volume of pDNA (plasmid DNA) solution with a transient induction of pores opening in cell membrane with a process called electroporation or electropermeabilization.[7] The advantage of pDNA vector is that it lacks of any viral component, hence with very low immunotoxicity.[7] However, its efficiency is lesser as compared to viral vector. In addition, there are naked DNA direct vector and oligonucleotides vectors with effectiveness similar to pDNA.

Another aspect of delivery of intended gene is that gene can be delivered directly in-vivo, or as frequently done ex-vivo from autologous hematopoietic stem cells (HSC), treated with correct gene, and re-injected back to the patient.[8]

2. Viral transfection method / gene-therapy through viral vectors.

Non-replicative recombinant viruses were first used as vectors in gene transfer to human cells.[9] Retroviruses and Adeno-associated viruses are most commonly used in the studies.[9]

- Retroviral vectors : The viruses are designed for capability of reverse transcription, but lack of replication capability. Gammaretroviral vectors were first shown to deliver genes into repopulating HSC.[10] Unfortunately, some of

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the patient treated by using gammaretroviral vectors also developed leukemia.[10] To overcome this side effect, self-inactivating (SIN) viral vectors are used. SIN vectors are devoid of long terminal repeat promoter/ enhancer function.[11] C-type retrovirus vectors were also used to deliver a normal copy of specific defective gene of T cells from patients with cancer or immunodeficiencies.[10]

- Lentiviral vectors: These vectors in contrast to retroviral vectors are able to transfer gene into non-dividing cells, but still can not reach the quiescent Go phase cells.[12] It can also carry larger and more complex gene package than gammaretroviral vectors, its development can provide advance in gene- editing of hemoglobinopathies.[13]
- Adeno-associated viral (AAV) vectors (Adenovectors): Adenovectors have been developed as gene delivery vehicle since early 80s.[14] Most earlier studies were based on human adenovirus serotype 5 (Ad5) and 2 (Ad2) of species C.[15] It is recognized that deleting the E1 and or E3 regions of their genomes rendered these vectors replication defective. [15] Other advantage, these vectors were able to infect both dividing and non-dividing cells.[15]
- Adenovectors could also efficiently enter into a variety of target tissues, such as liver, retina, cardiac muscle, and central nervous system, with specific tissue tropisms discovered in several naturally occurring adenovectors serotypes and those engineered with optimized capsids for tropism.[16]
- Disadvantages of viral vectors gene-therapy:
  - Can only do gene addition, and no other type of gene manipulations.
  - Potential of cellular and humoral immune response to viral antigen(s), most likely due to possible prior infection or exposure to the virus.

3. Zinc finger Nucleases gene-therapy.

Zinc finger nucleases (ZFNs) are a class of engineered DNA binding proteins that facilitate targeted editing of the genome by creating a double strand break in DNA, at the specific intended site. It consists of two functional domains:

- A DNA binding domain consists of a chain of two-finger modules, each recognizing a unique hexamer sequence of DNA. The two-finger modules are bind together to form zinc finger protein.
- A DNA cleaving domain consists of nucleases domain of FokI.
- When the DNA binding and DNA cleaving domains are fused together, a highly specific pair of genome scissor is created.[17]
- ZFNs have been engineered for gene modifications, targeting induction of insertion and deletion, gene correction of discrete base substitute specified by homologous donor DNA, and targeted gene addition. These modifications has been studied in human T cells, embryonic stem cells and cultured cell lines.[18]

4. TALENs gene therapy.

TALEs are transcription activator-like effectors that can be readily altered by attaching nucleases to become TALENs.[19] TALEs are proteins derived from plant pathogens, can be programmed to home in on specific DNA sequences. When attached to nucleases they can knock out a gene or change its sequence.[20]

Large sections of TALE protein consists of multiple sets of the same 34 amino acids occurring in tandem, except in the middle of each repeat there is a variable pair of amino acids. This constitute the 17-base stretch of DNA repeat in TALE.[21] TALENs is consists of a non-specific FokI nuclease domain that fused to customizable DNA-binding domain derived from TALEs.[22]

Application of TALENs for genetic editing is similar to ZFN. The simplicity with which TALENs can be designed together with its robust success rate has spurred the clinical adoption of TALENs as gene editing technology.[24]

TALE repeat arrays have been fused to transcriptional regulatory domain to create artificial transcription factor that can activate or repress gene expression, thus modulate expression of genes in plant and human cells, with changes in gene expression in the range of 2 – 30 folds.[23]

5. CRISPR Cas9 gene therapy.

CRISPR-Cas system is a natural adaptive immune system used by various bacteria and archaea to mediate defense against viruses and other foreign nucleic acid.[25] Prokaryotes can specifically incorporate short sequences from invading virus or genetic elements of plasmid into a region of its genome distinguished by clustered regularly interspaced short palindromic repeats (CRISPRs). When these sequences are transcribed into RNAs, they guide a multifunctional protein com-lex (Cas protein) to recognize and cleave incoming foreign genetic material.[26]

There are 6 types of CRISPR systems.[27] Type II is the most adopted because it is simple, efficient and with multiplexing possibilities. In this system, a combination of a single guide RNA (sgRNA), complementary to the DNA target site, and a Cas9 protein, which acts as a site specific nuclease, is used to generate a double-stranded DNA break at the selected sequence. Genomic target sequence binding by Cas9 and its cutting requires a PAM (protospacer adjacent motif) with sequence –NGG at the 3' site of the chosen target. Researchers from University of Illinois[28] have used CRISPR-Cas9 technology to develop a technology that can target any gene in the yeast Saccharomyces Cerevisiae, and turn it off by deleting single letter base from its DNA sequence. It is an advance of CRISPR- Cas9 technology to a single point precision.

CRISPR-Cas technology beyond gene editing.

- CAMERA or CRISPR-mediated analog multi-event recording apparatus.

Engineered cells are designed to record signals triggered by various stimuli using DNA of a "safe harbor" gene.[29] (Genomic safe harbors are sites in the genome able to accommodate the integration of new genetic material in a manner that ensures the newly inserted genetic elements are functionally predictable and do not cause alteration of the host genome) .

This tool could reveal how cells transition from stem to muscle or neuron and other cell types by harnessing the Cas9 protein to record cellular data on DNA. This tool is likely first to be used in researches to unveil cellular processes and signaling events.[29] Application in patient's cells, would still be a long shot.

- SHERLOCK or Specific high-sensitivity enzymatic reporter unlocking. This tool provides an inexpensive, easy to use, and sensitive diagnostic method for detecting nuclei acid material, such as virus, tumor DNA, and many other targets. In the new study, researchers use SHERLOCK to detect cell free tumor DNA in blood of lung cancer patients, and to detect synthetic Zika and Dengue virus.[30]
- DETECTR or DNA endonuclease targeted CRISPR trans reporter.

By combining Cas12a single-strand DNAse

activation with isothermal amplification, researchers create DETECTR , which can detect DNA with attomolar sensitivity, providing a simple but extremely sensitive platform for molecular diagnostics.[31]

- CRISPR-Cas9 Mutation prevention system.

Wyss Institute researchers developed an in vivo mutation prevention method that enables the Cas9 enzyme to discriminate between genomic target sites differing by a single nucleotide and to exclusively cut the unwanted one.[32]

Studies performed in E. Coli grown in vitro or in mouse GI tract. This approach can prevent the survival of antibiotic resistance variants.

- CRISPR as weapon of mass destruction.

The US Director of National Intelligence in his 2016 report : "Worldwide threat assessment of the US Intelligence community" called genome editing technologies like CRISPR a weapon of mass destruction.[33] According to MIT CRISPR could be used to make killer mosquitoes, plaques that wipe out crops, or snips at people's DNA. [33]

CLINICAL APPLICATIONS

1. Primary immunodeficiency diseases:

- a) X-linked severe combined immunodeficiency (SCID-X1) characterized by absence of T cells, impaired B cells function, lack of natural killer cells and gamma chain dependent cytokines. Using SIN gammaretroviral vectors to deliver a corrected copy of interlukin-2 receptor gamma chain (IL2RG) gene transduced ex-vivo autologous HSC to restore T cells population.[34]
- Adenosine deaminase deficient combined severe immunodeficiencies. (ADA-SCID). These patients have mutation in gene encoding adenine deaminase enzyme for clearance of toxic purine metabolites. In this disorder, patients can be treated with ADA enzyme replacement for life, or using viral vector to deliver the correct copy ex-vivo using autologoues HSC.[35]
- Wiskott-Aldrich syndrome (WAS).
- Patients with WAS has been treated the same way with correct gene copy of WASp. Therapeutic level of WASp correlate with clinical benefit of improvement from partial to complete resolution of bleeding, eczema, immunodeficiency and autoimmunity.[36]

2. Hematological disorders:

- Hemophilia B an X-linked clotting disorder is caused by lack of clotting factor IX (FIX) due to mutation in the FIX gene. FIX is produced in the liver. Induced pluripotent stem cells (iPSCs) are generated from patients' peripheral blood mononuclear cells, and using CRISPR-Cas9 to correct the gene defect. [37] After the correction, iPSC was directed to differentiate in vitro to become hepatic like cells[37]
- Beta-hemoglobinopathies. This group of disorders consists of sickle cell disease (SCD), and beta-thalassemia caused by mutation in the HBB (beta-globin) gene. Reactivation of developmentally silenced fetal HBG1 and HBG2 (gama-globin) genes is the goal for treating SCD and beta-thalassemia.[38] Hereditary persistence of fetal hemoglobin (HPFH) is a benign condition caused by point mutation in promoter region of the gamma-globin gene. Introduction of HPFH-associated mutations into erythroid cells by CRISPR-Cas9 disrupted repressor binding and raised gama-globin gene expression.[38]

3. HIV (Acquired immunodeficiency syndrome) :

HIV mainly infected the CD34+ T cells. A recent research has targeted to delete HIV from CD34+ T cells by gene editing techniques[39] targeting HIV-1 proviral DNA. The system includes a guide RNA specifically locates HIV-1 in T cell genome, and a nuclease to cuts the strands of T cell DNA. When the nuclease has edited out the HIV-1 DNA, the loose ends are reunited by cell's own repair mechanism.

4. Eye Disorders :

Voretigene neparvovec is approved recently by FDA for treatment of patients with bi-allelic RPE65 mutation-associated retina dystrophy that lead to vision loss. [40]

PRICE –TAG/ COST OF GENE THERAPY.

Alipogene tiparvovec, a therapeutic for patients with lipoprotein lipase deficiency (LPLD) that cause familial hyperchylomicronemia.[41] Patients with LPLD have very high triglycerides in their blood which can be toxic to pancreas and cause pancreatitis. AAV2 viral vector is used to correct the defective gene. Alipogene tiparvovec become the first approved gene therapy by Europe and US authorities. But, with a price-tag of Euro one million dollar for each therapy, we are wondering if that includes all the prior related research costs involved.[41] It could posts a challenge to standard reimbursement from government and or the private insurance companies.

CONCLUDING REMARKS

1. CRISPR-Cas9 technology could be the biotechnology of the "century", when we look at those research discoveries, such as CAMERA, SHERLOCK and DETECTR, especially CAMERA.
2. Gene expression and silencing are the result of many factors, pathways, and most importantly epigenetics through DNA methylation. The fact that TALENs, an easy to do technique, and relatively cheaper as compare to other gene-editing techniques, can also enhances gene expression, make it a potential tool for clinical application to control amount of gene(s) expression in certain conditions.It certainly would advance our understanding to many diseases and its treatment.
3. With continuous progress and refinements of technology, hopefully the cost can gradually be reduced.

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