Potential Application of Novel DNA Editing Techniques in Translational Neuroscience

In the past few years, in vitro and in vivo genetic manipulations and ultimately effective functional alterations of a gene product have achieved a turning point. Here, I briefly summarize this exciting news for scientists, clinicians and patients. These developments have brought about a great opportunity for everyone in research community particularly neuroscientists who study brain function. These technologies have paved the ground for the modification and correction of genomic DNA in patients who suffer from chronic conditions such as genetic disorders and cancer. These developments will also be very useful for scientists who study brain pathways to understand conditions such as mental retardations or addictions in animal models.

DNA editing techniques were developed to effectively alter a gene function at its DNA level or to study a gene activity by tagging its product and monitoring its expression and activity. These strategies were also faced multiple challenges such as their high costs, laborious and precision. Earlier methods such as mutation screenings, chemical mutagenesis or transposon mediated mutagenesis were all costly, lengthy and inefficient. Therefore the need to develop new strategies which could address all of the concerns received positive responses from scientists. Three of these new methodologies ZFP (Zinc Finger Protein), TALEN (transcription activator-like effector nuclease) and CRISPR (clusters of regularly interspaced short palindromic repeats) -mediated genome editing are the most promising in delivering effectiveness, precision, and feasibility in their potential applications in many clinical conditions particularly in neurological disorders (Gaj et al., 2013).

ZFP consists of the zinc finger DNA binding protein motives which bind specific DNA sequences, tagging a ZFP to either an endonuclease such as Fok I or transcriptional activator/repressor would transform the ZFP to a gene modifier. However, arranging these domains in a cloning vector in order to recognize a specific DNA sequence is costly and laborious. TALEN is based on the use of special effector proteins with specific DNA binding activities. TAL effectors were initially discovered in plant pathogens. They are introduced into plants by bacteria to specifically activate their gene transcription. The effectors are proteins and structurally contain N- and C- terminal and repeat domains of amino acid sequence which can specifically recognize a DNA sequence. Each repeat domain is different only in the amino acid number 12 and 13 which determine the specificity of the effector toward the nucleotides. By fusing the endonuclease Fok I to its C-terminal, TAL effectors are specialized into DNA editing tools. By pairing another specific TALEN, the two are able to recognize a specific DNA sequence and Fok I is able to catalyze a single cut at any specific site of double strand DNA. This will subsequently initiate DNA repair mechanism leading to the insertion or deletion of DNA nucleotides by non-homologous end-joining (NHEJ) (Wright et al., 2014).

A second promising technology to edit DNA sequences is recently developed. CRISPR consists of identical repeats and spacers sequences which have 100% homology to bacterial phages DNA. This allows the bacteria to actively recognize foreign DNA and destroys it. This mechanism constitutes the bacterial immune system. The CRISPR loci include multiple genes called Cas (CRISPR associated genes). Cas proteins are endonucleases and are able to generate double strand cuts on DNA molecules.

Using this principle, scientists have developed a state of art gene editing technology. The tool includes a CRISPR RNA product (crRNA) which is carrying a guide sequence (gRNA) and can form a loop with a transacting RNA, subsequently guiding Cas9 enzyme to a specific location on the genome where Cas9 makes a double cut on the DNA strand at a specific location. The result of the DNA breaks will either cause NHEJ by the cell repair

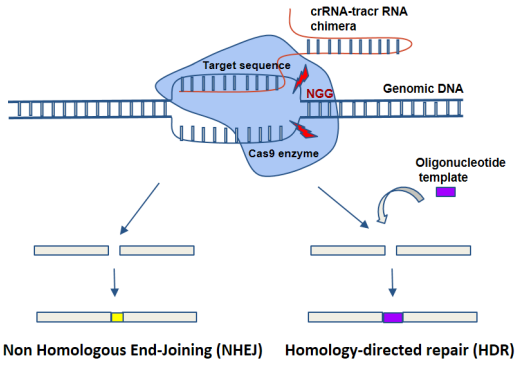

Figure 1. Schematic presentation of CRISPR/Cas9 technology and possible correction outcomes.

mechanisms or oligonucleotide-dependent homology-directed repair (HDR). HDR is considered an efficient and logical repair outcomes in correcting mutations in inherited disorders (Figure 1).

Currently, constructs were designed which include crRNA-transRNA chimera with or without Cas9 protein coding sequence. Using the specific DNA sequence designed as crRNA or gRNA, these constructs can be co-transfected into the desired cells or injected into one-cell embryos.

A number of CRISPR systems were developed including Cas9 nickase, a modified Cas9 protein which only catalyzes a single cut on one DNA strand. The application of two or four gRNAs will initiate a double strand cut at one position or at two positions on DNA, respectively. During a short period of time several laboratories have succeeded to apply this technology to a number of important applications such as generation of knockout mice using CRISPR technology or inserting an exogenous sequence into genome by in vitro and in vivo strategies or suppressing/reactivating gene expression (Hsu et al., 2014).

Although, this technology has clearly proved its versatility in modifying DNA sequences, some challenges are still ahead. For example, off-target efficiency is still high for human therapeutic applications even though it was shown in Cas9 nickase experiments that the efficiency has been enhanced and off-target cuts is greatly reduced, since one single strand off-target cuts would be promptly repaired by cell repair mechanisms. Another challenge is to increase the efficiency of Cas9. Cas9 is a bacterial protein and is not naturally expressed in eukaryotic cells. The DNA binding and targeting efficiencies are not 100%, therefore in many cells even the presence of Cas9 and the gRNA does not guarantee complete DNA break outcomes. These problems have challenged scientists to further improve Cas9 efficiency in Eukaryotic cells.

A very recent report has employed ZFP and TALEN methods to study the role of Fosb gene in addiction and depression induced changes in related brain areas by epigenetic remodeling. Cocaine induces Fosb expression in the nucleus accumbens (NAc), a brain area responsible for cognitive processing of motivation, pleasure and rewards. The fusion of ZFP with a repressor domain (Fosb-ZFP-G9a) to shut down Fosb promoter could repress its expression and therefore modulate addictive behavior in mice. The authors showed that the expression of Fosb-ZFP-G9a could enhance depression like behavior (Heller et al., 2014).

The application of CRISPR technology in hereditary and nonhereditary human health conditions were recently explored in animal models. The correction of dystrophin gene in animal model of Duchenne Muscular Dystrophy (DMD) using CRISPR/Cas9 technology was the first in its kind to correct a malignant gene and improve a disease condition in vivo. DMD is caused by mutations in dystrophin gene located on chromosome X. DMD patients suffer from muscle degeneration followed by weakness and atrophy and eventually die (Long et al., 2014).

The application of gene editing tools, particularly for CRISPR technology, in neurological sciences has just begun to be tested in in vivo to generate animal models of disorders or screen for anti-cancer drugs (Platt et al., 2014). We will hear a lot more about these exciting new technologies especially on CRISPR/Cas9, in near future. Like stem cell research and its applications in human health, it is predicted that CRISPR/Cas9 would develop into much more advanced technique with a direct clinical applications.

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