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Review

The application of genome editing in studying hearing loss

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ABSTRACT

Targeted genome editing mediated by clustered, regularly interspaced, short palindromic repeat (CRISPR)/CRISPR-associated nuclease 9 (Cas9) technology has emerged as one of the most powerful tools to study gene functions, and with potential to treat genetic disorders. Hearing loss is one of the most common sensory disorders, affecting approximately 1 in 500 newborns with no treatment. Mutations of inner ear genes contribute to the largest portion of genetic deafness. The simplicity and robustness of CRISPR/Cas9-directed genome editing in human cells and model organisms such as zebrafish, mice and primates make it a promising technology in hearing research. With CRISPR/Cas9 technology, functions of inner ear genes can be studied efficiently by the disruption of normal gene alleles through non-homologous-end-joining (NHEJ) mechanism. For genetic hearing loss, CRISPR/Cas9 has potential to repair gene mutations by homology-directed-repair (HDR) or to disrupt dominant mutations by NHEJ, which could restore hearing. Our recent work has shown CRISPR/Cas9-mediated genome editing can be efficiently performed in the mammalian inner ear *in vivo*. Thus, application of CRISPR/Cas9 in hearing research will open up new avenues for understanding the pathology of genetic hearing loss and provide new routes in the development of treatment to restore hearing. In this review, we describe major methodologies currently used for genome editing. We will highlight applications of these technologies in studies of genetic disorders and discuss issues pertaining to applications of CRISPR/Cas9 in auditory systems implicated in genetic hearing loss.

1. Introduction

Targeted genome editing defined as modification of the genome at a targeted locus, has long been used as a powerful tool to perform genome function studies in biomedical research. Previous methods such as gene knockdown with small interfering RNA (siRNA) and morpholinos have the potential drawbacks of off-targeting (Jackson et al., 2003; Fedorov et al., 2006; Robu et al., 2007) and incomplete editing (Holen et al., 2002; Elbashir et al., 2001; Bill et al., 2009). Conventional gene editing by homologous recombination (HR) can be used to modify genomes in various organisms (Adachi et al., 2006; Meyer et al., 2010; Rong and Golic, 2000). However, the

Abbreviations: AAV, Adeno-associated virus; ARHL, age-related hearing loss; Cas9, CRISPR-associated nuclease 9; CRISPR, clustered, regularly interspaced, short palindromic repeat; crRNA, CRISPR RNA; dCas9, defective Cas9; DSBs, double-strand breaks; gRNA, guide RNA; HDR, homology-directed-repair; HL, hearing loss; HR, homologous recombination; NHEJ, non-homologous-end-joining; PAM, proto-spacer-adjacent motif; RVD, repeat-variable di-residue; siRNA, small interfering RNA; TALENs, transcriptional activator-like effector nucleases; TALEs, transcription activator-like effectors; tracrRNA, *trans*-acting crRNA; ZFNs, Zinc finger nucleases

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extremely low efficiency of HR in mammalian cells (ranging from 1 in 10^8 to 1 in 10^5) limits its routine use (Reh and Vasquez, 2014). To overcome the shortcomings of these earlier methods, genome editing using programmable nucleases has become a promising alternative.

Three major programmable nucleases have been adapted as genome engineering techniques: Zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs), and CRISPR/Cas9. All three nucleases can be guided to induce site-specific DNA double-strand breaks (DSBs), which are repaired by both homologous and non-homologous mechanisms. Homologous repair enhances homologous recombination by at least two orders of magnitude (Rouet et al., 1994a, b), while non-homologous repair leads to targeted frame shift mutations (Bibikova et al., 2002). Applications based on the two DSB repair pathways lead to introduction of different types of mutations at target-specific sites of the genome including gene knock-out, knock-in, and point mutations.

Hearing loss (HL) is the most prevalent sensorineural disorder, affecting approximately 1 in 500 newborns (Mehl and Thomson, 1998). It is estimated that more than two-thirds of prelingual HL cases are found to be inherited, most of which are caused by mutations of a single gene that functions in the inner ear (Liu et al., 2001; Hilgert et al., 2009a,b; Morton and Nance, 2006). More than 150 chromosomal loci and over 80 genes have been identified to cause non-syndromic as well as syndromic forms of deafness (Yan and Liu, 2008; Hilgert et al., 2009a,b; Angel et al., 2012). The strong genetic basis of HL and spectacular advancements in CRISPR/Cas9-based genome editing technologies will surely usher in a new era using genome editing techniques to study HL, as can be glanced from our recent proof-of-principle study in the auditory system (Zuris et al., 2015). This review focuses on what we can learn from applications and challenges of CRISPR/Cas9-mediated genome editing and clinical therapeutic potential of CRISPR/Cas9 in the future, with implications in human genome editing in genetic HL. The current state of genome editing technologies will also be presented.

2. Comparisons among three genome editing techniques

2.1. Shared features of three genome editing techniques

All three programmable nucleases contain two functional domains: one is responsible for targeting and binding specific genomic sequence whereas the other is involved in inducing DNA DSBs (Fig. 1). For ZFNs, Cys2–His2 zinc fingers are amino termini of ZFNs, each zinc finger can be designed to recognize a three base-pair (bp) DNA sequence, and all zinc fingers (usually 3–6) are joined together to generate a single ZFN unit to target a DNA sequence that is 9–18 bp long (Miller et al., 1985; Wolfe et al., 2000; Urnov et al., 2010; Gaj et al., 2013). For TALENs, transcription activator-like effectors (TALEs) can also be modified to target a predetermined DNA sequence (Fig. 2). TALEs are naturally occurring proteins derived from plant pathogenic bacteria *Xanthomonas* spp., most of which contain 13–28 repeats and 33–35 amino acids per repeat (Boch and Bonas, 2010). Each repeat can recognize a single nucleotide in the target sequence, and the nucleotide specificity is determined by a hypervariable region of two adjacent amino acids at positions 12 and 13 within each amino acid repeat, termed repeat-variable di-residue (RVD) (Moscou and Bogdanove, 2009; Boch et al., 2009; Morbitzer et al., 2010; Streubel et al., 2012; Cong et al., 2012). Multiple TALEs repeats are joined in tandem to target a specific DNA sequence (Bogdanove and Voytas, 2011). For CRISPR, the repeats are derived from prokaryotic RNA-guided adaptive immune system, which are transcribed as target-specific CRISPR RNA (crRNA) and *trans*-acting crRNA (tracrRNA). TracrRNA is essential for the maturation of crRNA and can team up with crRNA guiding Cas9 nuclease to cleave invading phages or plasmids by binding to complementary DNA sequence in viruses or plasmids and inducing DSBs (Jinek et al., 2012; Mali et al., 2013; Cho et al., 2013).

All three systems can induce DNA DSBs: ZFNs have non-specific DNA nuclease – FokI, FokI nucleases must dimerize to be active to induce DNA DSBs (Vanamee et al., 2001). For CRISPR, type II CRISPR-Cas system is adapted as a genome editing tool, in which

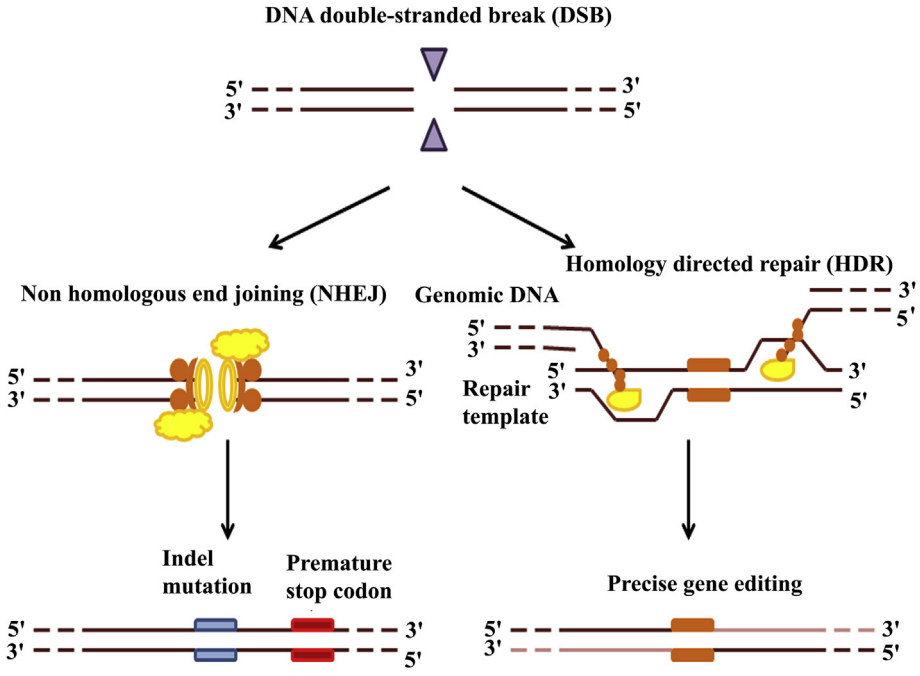

Fig. 1. A schematic representation of programmable nucleases demonstrating two functional domains.