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Cut and paste the genome: Genome editing for research and therapy

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Abstract. Genome engineering, or alternatively called 'genome editing,' has been one of the rapidly growing fields of biotechnology for the last few decades. Scientists are now making targeted modifications of genome in any organism of choice with improved precision. In this mini review, we provide basic, fundamental theory and mechanisms of the well-known genome editing technologies such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regulatory interspaced short palindromic repeats (CRISPR)/Cas9 system. We also discuss its role in the field of genetic research and highlight its therapeutic potential as an indispensable tool for the development of personalized medicine to come in near future.

1. Introduction

For the last few decades, the biotechnology to decipher individual human genome such as whole-genome sequencing (WGS, [1]) and whole-exome sequencing (WES, [2]) advanced greatly, lowering barriers to decoding the information embedded in human genome. Such technological advances are anticipated to revolutionize our understanding of clinical genetics and to deliver personalized medicine in near future. Nevertheless, the present challenge lies in converting this tremendous amount of data (i.e., billions of nucleotides that comprise DNA of an individual) into clinically relevant information, such as how genotype affects phenotype. To transform the data into functionally relevant knowledge, technologies such as targeted gene inactivation by homologous recombination [3] or targeted gene knockdown by RNAi [4] have been utilized as means of providing empirical information to elucidate the function of genes of interest. However, the former has limitations such as extremely low efficiency of correct insertion into the target chromosome (1 in $10^6 \sim 1$ in 10^9) [5] and potential adverse mutagenesis [6], while the latter has limitations including incomplete and temporary knockdown of target gene and undesired off-target effects [7]. More recently, genome engineering technologies, commonly referred to as 'genome editing,' have been emerged, enabling scientists to make targeted modifications to the genome in practically any organism of choice with improved precision [7a]. This technology utilizes engineered nucleases which

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are complex of sequence-specific DNA binding domains and nonspecific DNA cleavage modules [8]. In this brief review, we aim to provide basic information on this genome editing technologies and discuss the applications and the therapeutic potential of these technologies, as well as future prospects.

2. Principle mechanisms of genome editing technologies

Genome editing technology involves customized programmable DNA-binding nucleases such as zinc-finger nucleases (ZFNs) [8], transcription activator-like effector nucleases (TALENs) [9], and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 (CRISPR-associated 9) nucleases [10]. Although each nuclease has unique mode of action, in principle, these nucleases recognize, bind to, and cleave chromosomal DNA to create site-specific DNA double strand breaks (DSBs). Subsequently, these DSBs trigger endogenous DNA repair systems, such as homology directed repair (HDR) or error-prone non-homologous end joining (NHEJ), resulting in targeted genome modification (Fig. 1) [11].

3. Programmable DNA-binding nucleases

3.1. Zinc-finger nucleases (ZFNs)

First discovered in transcription factor from *Xenopus laevis* [12], Cys2-His2 zinc-fingers are the most common sequence-specific DNA-binding motifs found in all eukaryotic organisms [13]. Each zinc-finger

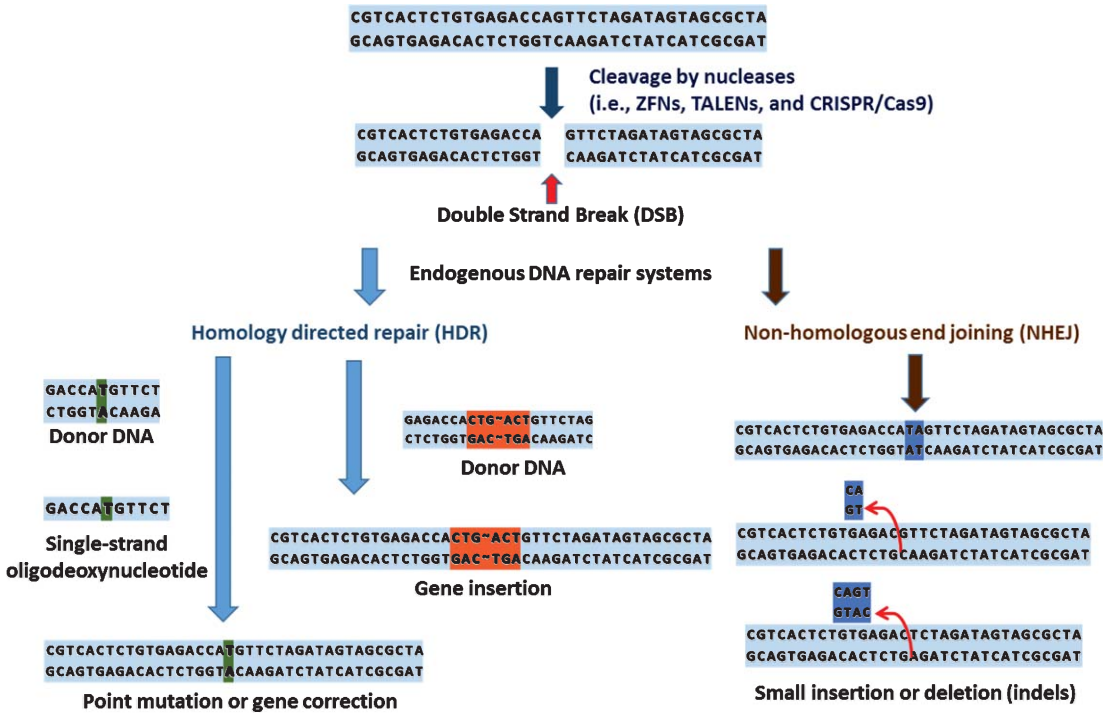

Fig. 1. Schematics of genome editing process.

motif consists of about 30 amino acids in a ββα configuration, and two cysteines and two histidines coordinate a single zinc atom in individual zinc-finger [14]. Amino acids in positions −1, 3, and 6 on the α-helix contact 3 base pairs in the major groove of DNA [15]. Thus, theoretically, a combination of 6 separate zinc-fingers that each recognizes a 3 base pair DNA sequence can consequently recognize a specific DNA sequence of 18 base pairs, which is long enough sequence to specify a unique site in the human genome. The construction of such unnatural, synthetic zinc-fingers that recognized 18 base pairs of DNA sequence made application of zinc-finger proteins for recognizing specific DNA sequence possible [16]. However, it was the combination of zinc-finger proteins with cleavage domain of endonuclease that made possible to realize the concept of targeted genome engineering. Isolated from *Flavobacterium okeanokoites*, the type II restriction endonuclease Fok I recognizes the DNA sequence of 5'-GGATG-3' and cleaves 9th and 13th base pairs from the recognition site, regardless of the sequence at the cleaved site [17]. When the cleavage domain of Fok I endonuclease was combined with zinc-finger protein to generate zinc-finger-Fok I fusion proteins, these hybrid fusion proteins (ZFNs) were able to cut DNA at the predetermined sequences [18].

Since Fok I cleavage domain must dimerize to catalyze DNA cleavage, during ZFN-mediated site specific DNA cleavage, ZFN target sites consisted of two zinc-finger binding sites separated by 5∼7 base pairs of spacer sequence are occupied by two ZFNs on each strand and the cleavage domain of each ZFN dimerize on the spacer sequence and cleaves it (Fig. 2). Currently there are 3 large sets of constructed ZF proteins (ZFPs) from independent groups, namely The Scripps Research Institute [19–21], Sangamo Biosciences [22], and ToolGen [23], are available. Recently published articles on the use of ZFNs are listed in Table 1. The contents of Table 1 are not based on scientific significance by any means. It is just examples of recent research trend involving use of ZFNs. As demonstrated in the Table below, researches on ZFNs are still active, and most of the cases the ZFNs are utilized as a tool for studying function of

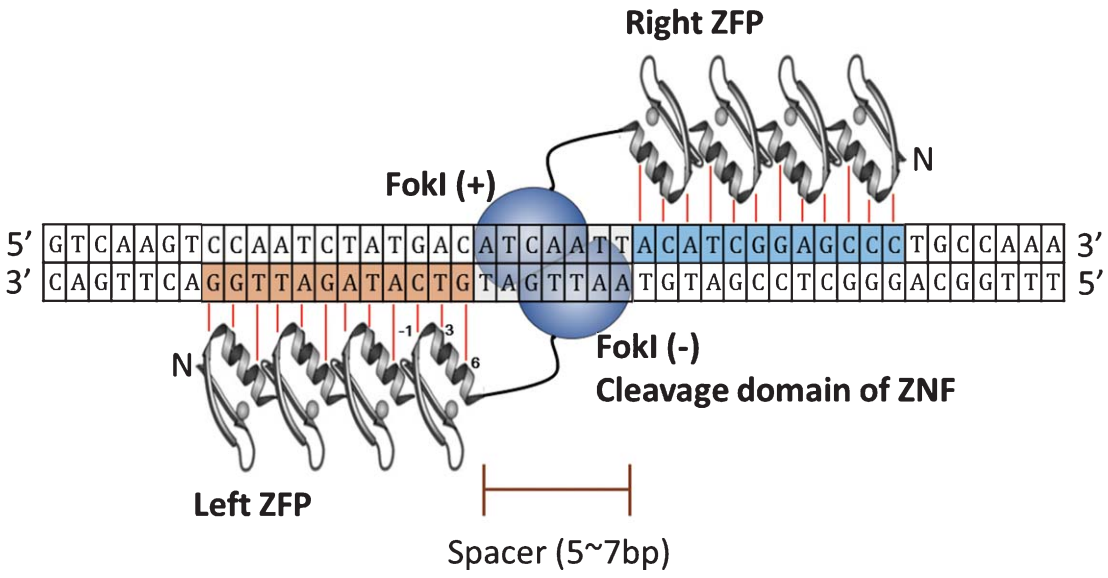

Fig. 2. ZFN dimerize on the spacer sequence via sequence-specific interaction between ZFP and DNA. Amino acids in positions −1, 3, and 6 on the α-helix recognizes and contact 3 base pairs in the major groove of DNA. FokI cleavage domains from each ZFN dimerize over the spacer sequence to facilitate DBS.

Table 1
Selected recent examples of ZFN-mediated genome editing in various organisms including human cells

Gene modification	Organism	Genes	Refs
Disruption	Mouse	tbc1d20, rab3gap1, rab3gap2, rab18	[24]
	Sheep	mstn	[25]
	Human	MDR1, MRP2, BCRP	[26]
	Human	HPV E7	[27]
	Humanized mouse	CCR5	[28]
Addition	Cow	hLYZ	[29]
	Human	CCR5	[30]
Correction	Human	AAVS1	[31, 32]
	Human	HBB	[33, 34]

Few selected, recent articles searched through Pubmed. The literature search was to overview the research trend regarding ZFN-mediated genome editing.

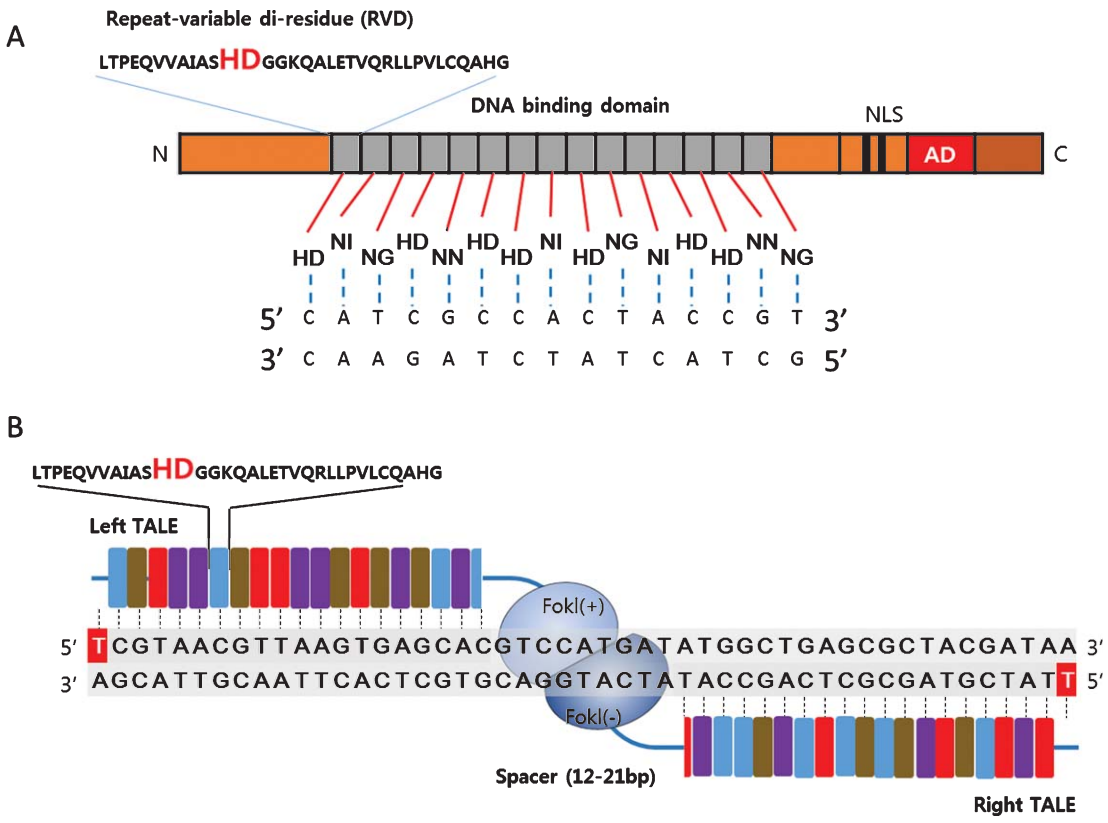

Fig. 3. Structures of TALE and TALEN. A. Schematic presentation of TALE (TAL effector). Each TALE repeat contains 33–35 amino acids, and each RVD (amino acids at positions 12 and 13) recognizes a corresponding single base pair. RVD within a consensus repeat sequence is indicated in red. NLS: nuclear localization signal, AD: transcriptional activation domain. B. TALEN pair binds to specific DNA sequence recognized by TALEs. FokI cleavage domains from each TALE dimerize over the spacer sequence. In most cases, naturally occurring TALEs are preceded by T at the 5'-end [39] as indicated by red box.

certain genes by inducing gene disruptions. However, there are also studies to correct mutations in human cells, implicating the therapeutic potential of genome editing using ZFNs.

3.2. Transcription activator-like effector nucleases (TALENs)

TAL (transcription activator-like) proteins were first identified as secreted proteins from bacterial plant pathogen *Xanthomonas*, and the named based on observation that they activated transcription of endogenous pathogenic genes in plants [35, 36]. Once they enter the nucleus of host cells, they bind to effector-specific sequences on the promoters of host genes to initiate transcription [37]. TALENs are composed of DNA-binding domain (DBD), N-terminal, C-terminal, and FokI endonuclease domain. The DBD of TALEN is composed of 'repeats' that itself composed of 33∼35 amino acids [38].

Among these amino acids, the amino acid residues at position 12 and 13 of each repeat decide which nucleotide of DNA they bind to (i.e., Asparagine-Isoleucine (NI) ⟶ Adenine (A), Asparagine-Glycine (NG) ⟶ Thymine (T), Aspargine-Asparagine (NN) ⟶ Guanine (G), Histidine-Aspartic Acid (HD) ⟶ Cytosine (C)) so they are named as 'repeat-variable di-residue (RVD) [39]. It is this one RVD to one nucleotide match between the RVD of TALENs and chromosomal DNA that underpins target sequence specificity of TALENs (Fig. 3A). Furthermore, this sequence specific interaction and simplicity of its coding system made generation of customized TALE domains that target DNA sequence of interest possible [40, 41].

The TALE domains can be assembled in predetermined combinations and linked to the Fok I nuclease to drive sequence-specific DSB of chromosomal DNA [42]. TALENs, in pairs, bind to opposing target sequences so that the linked Fok I nucleases come in contact with the spacer from opposite sides. The

Table 2
Selected recent examples of TALEN-mediated genome editing in various organisms including human cells

Gene modification	Organism	Genes	Refs
Disruption	Mouse	c-kit, PU.1	[43]
	Zebrafish	nptx2a	[44]
	X. tropicalis	TRα	[45]
	Human	Rap1	[46]
	Human	Gads	[47]
	Zebrafish	rspo2	[48]
	Mouse	ace2	[49]
	Zebrafish	fshb, lhb	[50]
	X. tropicalis	dot1l	[51]
	Human	miR-21	[52]
Addition	Human	eGFP	[53]
Correction	Human	DMD	[54, 55]
	Human	CCR5	[56]
	Human	HBB	[57–59]
	Mouse	Crb1	[60]
	Human	XPC	[61]

Few selected, recent articles searched through Pubmed. The literature search was to overview the research trend regarding TALEN-mediated genome editing.

Fok I nucleases from each TALEN forms Fok I dimer and it cleaves double-stranded DNA (Fig. 3B). As indicated in the Table 2, TALEN-mediated genome editing is also widely used to facilitate gene disruption, addition, and correction.

3.3. Clustered regulatory interspaced short palindromic repeat (CRISPR)/Cas9

The existence of CRISPR was first discovered by Nakata et al. in studying *iap* gene of *E.coli* [62]. What they found was 29 nucleotide-long repeats interspaced by 5 intervening 32 nucleotide-long, non-repetitive sequences. The actual word CRISPR for describing such repeat sequences was first coned by Jansen in 2002 [63]. The CRISPR loci are composed of a set of CRISPR-associated (Cas) genes and a series of repeats (direct repeats, approximately 20–50 base pairs) interspaced by unique, non-repetitive sequences (spacers).

These non-repetitive sequences correspond to the sequences of foreign genetic elements (protospacers) [64]. the protospacers are flanked by a short protospacer adjacent motif (PAM) that is either located on the 3' (type II CRISPR) or 5' (type I CRISPR) of foreign DNA [65]. Additionally, leader sequence, rich in A+T sequences, is known to serve as a promoter element for the CRISPR loci [66] (Fig. 4). CRISPR is an essential components of RNA-based adaptive immune systems of bacteria and archaea [67]. In response to foreign genetic element challenges, bacteria and archaea incorporate short fragments of foreign genetic elements into host genome as shown in Fig. 4. When CRISPR locus is transcribed, the long primary transcript is processed to produce a library of short CRISPR-derived RNAs (crRNAs) [68, 69]. Each crRNA has a complementary sequence to a previously encountered foreign genetic elements, and it mediates detection and subsequent destruction of foreign nucleic acids [70]. This unique immune system can be divided into 3 distinct CRISPR types (type I-III) based on gene conservation and locus organization [71]. Especially in the type II CRISPR/Cas system, crRNAs hybridize with trans-activating crRNAs (tracrRNAs) to facilitate RNA-guided sequence-specific DNA cleavage by Cas9 proteins. Cas9 utilizes RNA-DNA pairing to target foreign DNA, and Cas9-RNA complex-mediated DNA cleavage requires recognition of PAM where DNA strand separation and RNA-DNA hybrid formation occurs (Fig. 5) [72].

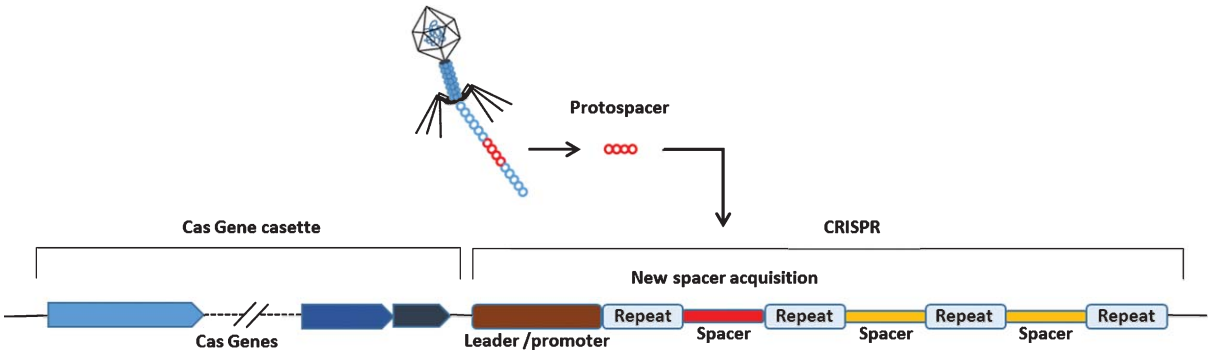

Fig. 4. Schmatics of a generalized CRISPR locus. Upon introduction of foreign genetic elements from bacteriophages or plasmids, Cas proteins obtain spacers from the exogenous protospacer sequences and they are incorporated into the CRISPR locus of host genome.

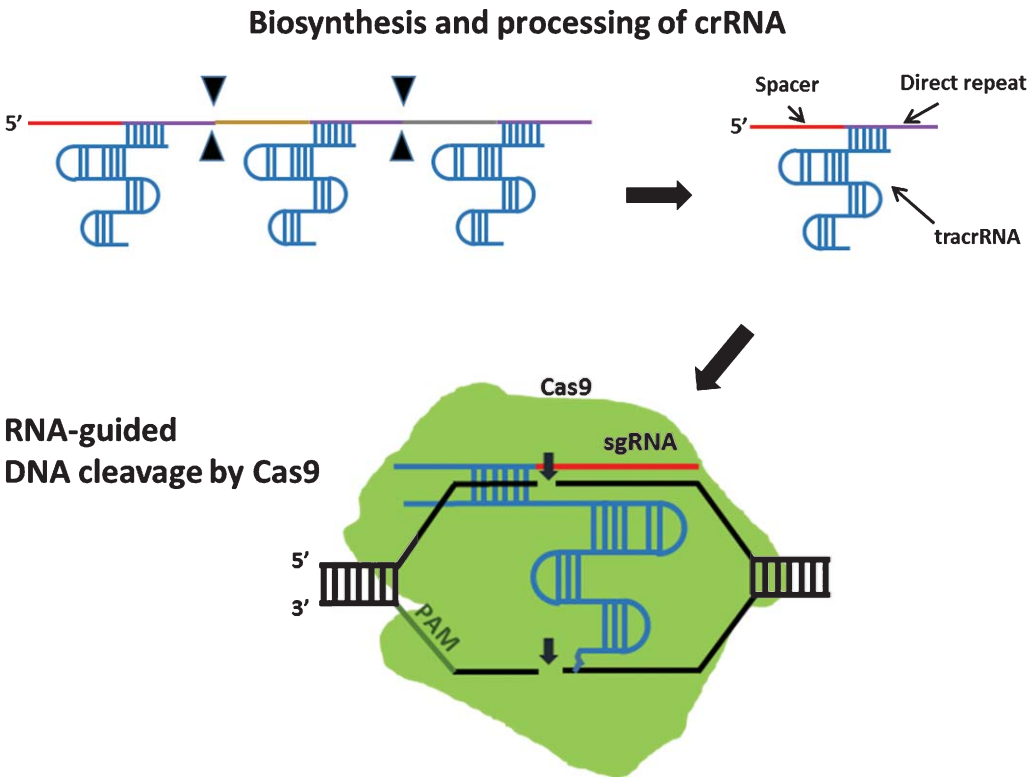

Fig. 5. DNA cleavage by type II CRISPR nuclease system. In type II CRISPR, crRNA-tracrRNA hybrids complex with Cas9 to facilitate sequence-specific DNA cleavage. Filled triangle indicates cleavage by Cas ribonucleases. Small arrows indicate DNA cleavage by Cas9. sgRNA: single guide RNA.

Table 3
Selected recent examples of CRISPR/Cas-mediated genome editing in various organisms including human cells

Gene modification	Organism	Genes	Refs
Disruption	Mouse	PrP	[73]
	Human	CCR5	[74]
	human	PVALB, EMX1	[75]
Addition	Human	AAVS1	[76]
Correction	Mouse	Crygc	[77]
	Human	DMD	[54]
	Mouse	DMD	[78]

Few selected, recent articles searched through Pubmed. The literature search was to overview the research trend regarding CRISPR/Cas-mediated genome editing.

DNA sequence. In fact, reprogrammed RNA-guided nucleases have been demonstrated to be competent in facilitating gene disruption, addition, and correction in human cells and other model organisms (Table 3).

Compared to ZFNs and TALENs, CRISPR/Cas9 system has higher efficiency and shortest target length, and it can also facilitate multiplex targeting [79]. Furthermore, Cas9 can be used for applications other than usual category of gene modifications (disruption, addition, and correction), such as, but not limited to, transcriptional control or DNA labeling [80, 81].

4. Perspectives: The role of genome editing in achieving personalized medicine

Although it is in its infancy, over the last few decades, genome editing has come of age and tremendous interest has been placed on its utility as a tool for basic/clinical genetics. Accumulating data also indicate its potential as a dexterous and powerful means of personalized medicine which is expected to provide individual patient with 'tailored' therapeutics near future. Promising results using these site-specific nucleases in therapeutic approaches against severe combined immune deficiency (SCID) [82], sickle cell disease [83], and hemophilia B [83], make the hopes of personalized medicine up ever more. For 'personalized medicine' to be realized, close cooperation of related biotechnologies and disciplines is required (Fig. 6).

For example, an ideal starting point of personalized medicine would be genomic information analysis of an individual patient. Biotechnologies such as WGS and WES would be a valuable tool for this task. From

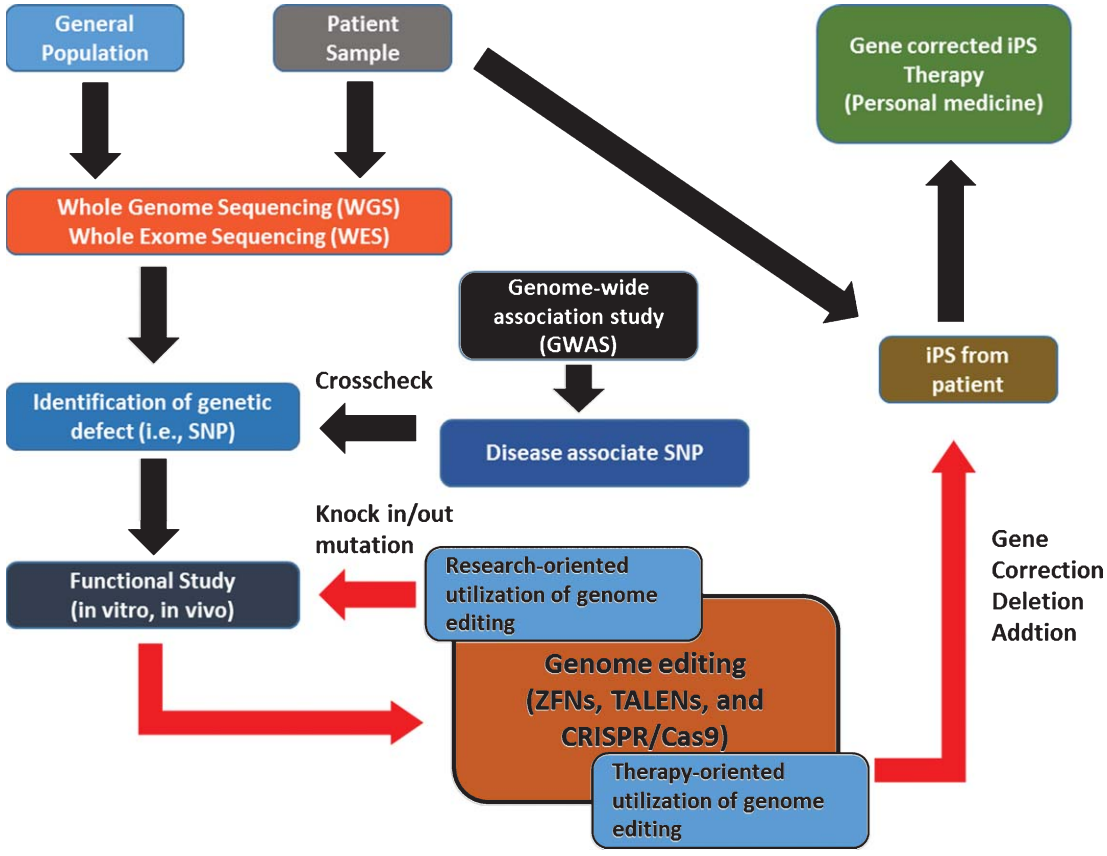

Fig. 6. Example of genome editing utilized in personalized medicine.

the genomic analysis by sequencing can identify specific genetic defects of patients (i.e., single nucleotide polymorphism, SNP), and the relationship between specific SNP and phenotypic manifestation can be systemically examined both *in vitro* and *in vivo*. At this stage comes the genome editing technology. With the genome editing technology (i.e., gene knock in/out, point mutation, gene correction, and etc.) the functional consequences of a certain SNP can be determined in details (research-oriented utilization of genomic editing). Furthermore, genomic editing can serve as a part of therapeutic process that requires genomic modifications (i.e., gene correction of induced pluripotent stem cells (iPS) from specific patient). Thus, the genome editing technology can be both research-oriented and therapy-oriented applications. Although there are still some unsolved issues regarding detailed mechanisms of individual genome editing such as how foreign sequences are selected and incorporated into the CRISPR loci of host, it is undoubtful that these technology eventually revolutionizes the field of genetic research as well as our understanding of diseases and opens a new paradigm in fighting currently incurable diseases.

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Conflicts of interest

The authors declare no conflict of interest.

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