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Widely used gene editing strategies in cancer treatment a systematic review

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ABSTRACT

Cancer is considered one of the most dreaded diseases all over the world. Nearly half of the cancer patients diagnosed are dying throughout the year. Despite the advancement in the last years, the high cancer mortality rate reveals the urgent requirement of more effective remediation. Gene editing is a new technology capable of deletion, mutation, or substitution of target genes and has a great possibility for treating cancer. Gene editing using engineered nucleases has enabled researchers to specifically modify genes for various applications such as biotechnology and gene therapy. However, the most common engineered nucleases used in genome editing technology are zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats associated RNA guided Cas9 (CRISPR-Cas9) nucleases. The genome-editing process utilizes those nucleases to generate a DNA double-strand break (DSB) and then allow the repair machinery of the cell to fix the break. Consequently, the DNA sequence is changed precisely. In the present review, we explained the technicality of gene editing with engineered nucleases exploited to identify the target genes for treating cancer.

1. Introduction

Cancer has been considered as one of the main causes of disease-related mortality, with an ascendant occurrence worldwide (Torre et al., 2015). Despite the development in treating cancer, cancer-related death rates stayed comparatively steady (Hoyert, 2012). The classical and modern patterns used for cancer therapy such as surgery, chemotherapy, radiotherapy, immunotherapy, and targeted therapy, have had negative effects on the quality of life. Therefore, the effort to find more efficient and more tolerable anticancer treatment continues (Palti, 1962). In recent decades, targeted therapies have brought hope for treating numerous cancer types. However, in many patients, the drugs ultimately stop working. The reason for that is not only the complex pattern of mutagenesis in tumors but also the heterogeneity within the microenvironment (Vogelstein et al., 2013).

Cancer is described as a cumulation of many genetic and epigenetic changes in the genome of cancer cells. This leads to pathogenesis and the development of cancer to disturb cellular signaling and bring about tumorigenic transformation and malignancy (del Sol et al., 2010). Accordingly, the capability of correcting or disabling specific regions in the genome of a cancer cell can provide an interesting modality to treat cancer diseases. This approach can be accomplished by genome editing (Yi and Li, 2016). Meanwhile, the advancement of designed nucleases including activator-like effector of transcription nucleases (TALENs) and nucleases of zinc-finger (ZFN) has allowed researchers to immediately target and change the genetic sequence (Joung and Sander, 2013; Urnov et al., 2010).

Recently, genetic engineering has been rapidly developed by the progress of the CRISPR/Cas9 system. Since it was first used as a gene-editing method in mammalian cells in 2013 (Cong et al., 2013; Mali et al., 2013), CRISPR technology has been constantly being developed, enabling both alterations of the genetic information of cells and organisms, and also the creation of epigenetic and transcriptional changes (Zhan et al., 2019). Gene editing techniques rely upon generating a double-strand break (DSB) in a specific section of the genome and repairing that break by cellular processes. Unlike earlier approaches for

Abbreviations: AAV, Adeno-associated virus; CRISPR-Cas9, clustered regularly interspaced short palindromic repeats associated RNA guided Cas9; crRNA, CRISPR RNA; DSB, double-strand break; HDR, homology-directed repair; IDLVs, Integrase-deficient lentiviral vectors; MCM10, minichromosome maintenance 10; MERS-CoV, Middle East respiratory coronavirus syndrome; NHEJ, non-homologous end joining; SARS-CoV, severe acute respiratory coronavirus syndrome; TALENs, transcription activator-like effector nucleases; tracrRNA, transactivating CRISPR RNA; ZFNs, zinc finger nucleases.

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gene editing, zinc-finger nuclease, and transcription activator like-effector nuclease, CRISPR method has been quickly and extensively adopted by researchers as it is simple, scalable, available, and it has made a significant progression in the gene-editing field (Barrangou et al., 2007).

2. Therapeutic appliances of gene editing

Numerous diseases such as hematologic cancers are triggered by somatic or germinal mutations. Germinal mutations take place in the germ cells which eventually mature into reproductive cells (ovum and sperm) (Erickson, 2010). Hence, if the mutation occurs in the cells that participate in fertilization, it could be carried from parents to their offspring. As for somatic mutations, they are received and segregated to particular cells (Furutani and Shimamura, 2017). So far, gene therapy has been advanced for only applications that target somatic mutations owing to the debatable topic for ethical argument about germline modifications (Mussolino et al., 2017).

However, the capacity for correcting or disabling mutations that induce cancer and other genetic disorders is an interesting and intractable therapeutic strategy. Fortunately, designing the engineered nucleases for editing genome has made that a reality. Owing to having distinct properties, every nuclease can be better suited for a certain application against others. Generally, each particular nuclease composes of a specific domain which specifically binds to the desired sequence and a nonspecific domain (Hilton and Gersbach, 2015).

3. Zinc finger nuclease

Zinc finger nuclease (ZFN) is a chimeric nuclease comprised of a specific domain that binds to DNA and a nonspecific *Fok*I endonuclease. The specific domain is made up of many zinc finger protein motives that have specificity for the nucleotide triplet (Fig. 1) (Carroll, 2011). Various zinc finger protein motives can be connected so that they can bind to a more extended specific DNA sequence. The DNA-related domain typically consists of three to six zinc finger protein motives which identify nine to eighteen base pairs in DNA, also it is possible to be modified to recognize certain sequences in DNA double-strand (Urnov et al., 2010; Scott, 2005).

Nonspecific cleavage of DNA is done by the *Fok*I endonuclease which demands dimerization to be activated. Thus, a pair of ZFNs, linked to a monomeric cleaving domain, is engineered for binding the opposite DNA strands (Szczepek et al., 2007). The binding of those ZFNs with the target DNA gives rise to dimerization and DNA breaking. Due to the requiring for joining of two proteins which bring about dimerization, ZFN method has more specificity to the target sequence. Therefore, this technology has been eligible for purposes of genome editing (Miller et al., 2007).

In fact, Zinc finger nuclease has been utilized to target numerous forms of cancer. ZFN-mediated knockout of HAb18G/CD147 has resulted in a considerable decrease in the abilities of cell adhesion, migration, invasion, and colony formation of *in vitro* hepatocellular carcinoma cells (Li et al., 2015). Moreover, it has been shown that using ZFN-based MALAT1 knockout, that the complete loss of MALAT1 has no effects on cell proliferation of liver and lung cancer cells (Eißmann et al., 2012). The researchers also have targeted the mTOR locus to inhibit PI3K/Akt/mTOR pathway, which is required for the development and growth of breast cancer cells, then, it can provide possible remediation for that cancer (Puria et al., 2012). Sun and his colleagues 2017 in their study demonstrated, by deleting MIIP gene using ZFN technology, that haplo insufficiency of MIIP increases the development, migration, and invasion of colorectal cancer cells *in vitro* and *in vivo* (Sun et al., 2017). This technique was also used to target genes for MCT4 and BASIGIN in the cell lines of colon adenocarcinoma and glioblastoma; thus, it might be an efficient strategy for inhibition of glycolytic tumors (Marchiq et al., 2015).

4. Transcription activator–like effector nucleases

Like ZFN, Transcription activator-like effector nuclease (TALEN) is a

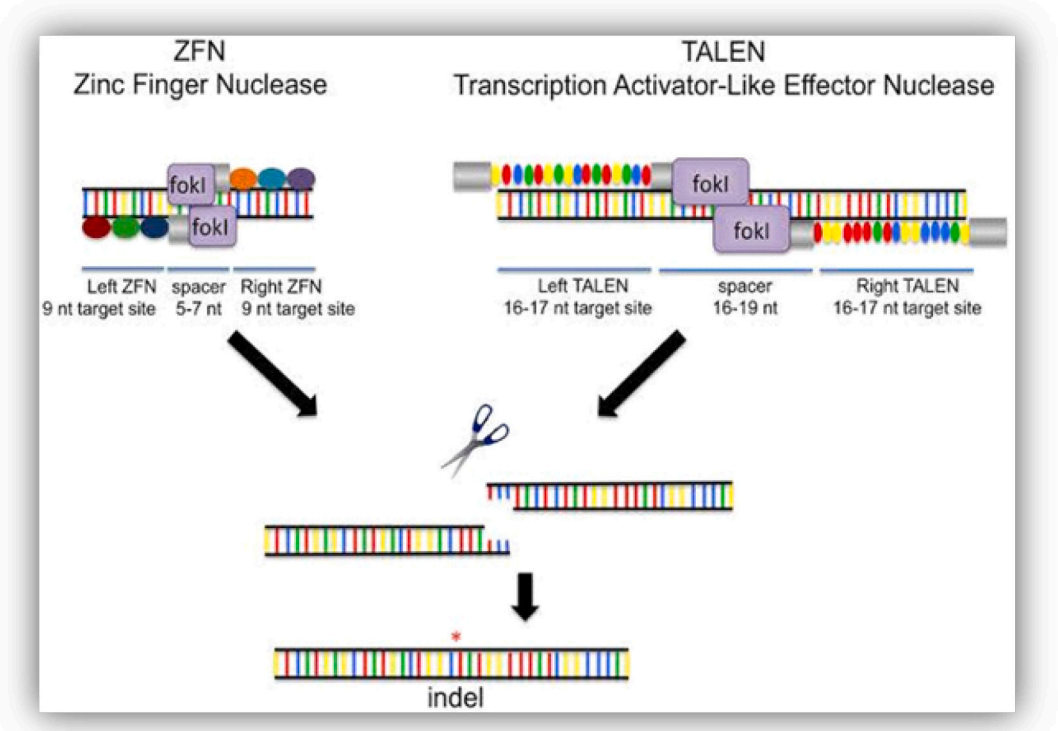

Fig. 1. Shows the mechanism of ZFN and TALEN (Moore et al., 2012).

chimera consisted of a specific DNA linking a nonspecific *Fok*I nuclease and domain. It also involves dimerization to activate FokI nuclease and induce the target DNA sequence cleavage (Fig. 1) (Joung and Sander, 2013). The main difference between TALEN and ZFN is the domain that binds to DNA. TAL proteins are made up of highly preserved sequence contains between 33 and 35 amino acids which are different solely at the location 12 and 13, a reiterated inconstant double-residue region. Thus, TALENs have consisted of an order of TALE repeats (Bogdanove and Voytas, 2011), which causes TALEN to be larger in comparison with ZFN and possibly more difficult to deliver. On the other hand, TALEN can recognize single nucleotides more specifically in contrast to ZFN technology (Cuculis and Schroeder, 2017).

In addition, TALEN has been exploited to target several genes in cancer cells. It has been concluded, by TALEN-mediated Cdh17 knockout, that cadherin-17 has a vital role in suppressing intestinal tumor growth (Chang et al., 2018). Lee and his team work 2015 (Lee et al., 2015) investigated that TMSB4X knockout mediated by TALEN has resulted in reduced metastasis in lung cancer cells, since Tβ4, an actin-sequestering protein, is essential for cancer cell migration. In order to investigate their efficacy in eliminating cervical cancer cells, TALENs have also been used for targeting E7, an oncoprotein accountable for malignant transformation in cervical cancer (Shankar et al., 2017).

In addition, by TALEN-based girdin knockout, it has been proposed that girdin is crucial in controlling the formation, invasion, and migration of esophageal carcinoma cells (Cao et al., 2014). Moreover, PIWIL2 knockdown, using this technique, has been demonstrated to suppress cell proliferation in hepatocellular carcinoma (HepG2) cells (Chen et al., 2014). Nrf1α knockout was also conducted in HepG2 resulting in a significant increase in the capacities of invasion and migration of the cells (Ren et al., 2016).

5. Clustered regularly interspaced short palindromic repeat (CRISPR) and CRISPR-associated protein nucleases

CRISPR and CRISPR-related protein 9 nuclease (Cas9), produced by *Streptococcus pyogenes*, has recently been widely exploited as strategies in genome editing by the research and also the clinical perspective (Gasiunas et al., 2012; Jinek et al., 2012). Unlike the previous nucleases, the CRISPR-Cas9 technique produced by the adaptive immune system in prokaryotes utilizes RNA to detect target regions in the genome to be cleaved. Upon invading bacterial cells, viral genetic material could be integrated into a CRISPR position to be transcribed into CRISPR RNA (crRNA) which eventually makes a complex with trans-activating crRNA and Cas9 endonuclease (Doudna and Charpentier, 2014). Each of two nuclease parts of Cas9, HNH-like nuclease, and RuvC-like nuclease cleaves one DNA strand, which results in ds-DNA blunt end cut at the location of a specifically intended sequence incorporated with crRNA that is made up of 20 nucleotides and complements to DNA desired sequence. However, the target DNA requires a protospacer-related motif sequence for being recognized (Sapranauskas et al., 2011).

The CRISPR-Cas technology was firstly composed of the three components mentioned above, but now, due to intensified investigations, CRISPR-Cas9 technique has been modified to be composed of two components, Cas9 nuclease which motives the cleavage of DNA and guide RNA that is a combination of crRNA and trans-activating crRNA (Jinek et al., 2012).

In addition to the system described above, two other various CRISPR-Cas9 techniques were discovered. The Cas9D10A system combines with nickase instead of nuclease which generates a single-strand DNA break, affecting the cellular DNA repair system (Fig. 2) (Cong et al., 2013). As for the last system, in spite of DNA binding, DNA strands are not cleaved as this system does not have Cas9 nuclease (dCas9) (Qi et al., 2013). In general, CRISPR-Cas9 nucleases are not difficult to be designed and manipulated, as opposed to the other genome editing systems. Modification of target specificity requires only redesign of the crRNA sequence; thus it is much easier compared with modifying target specificity regarding ZFN and TALEN. Moreover, the CRISPR/Cas technique can be implicated with multiple guide sequences, which allows editing more than one locus in the genome concurrently (Barrangou, 2014).

The Cas9 nuclease produced by *S. pyogenes* is the most extensively used in spite of doing further modifications in the system by Cas9 or Cpf1 nuclease produced by *Staphylococcus aureus* and Francisella species, respectively (Zetsche et al., 2015; Fagerlund et al., 2015). CRISPR/Cas9 technology has been utilized for identifying possible remedial targets in numerous cancers. In breast cancer cells, it has been found that CRISPR-mediated knockout of MIEN1, migration, and invasion enhancer 1, does not affect the morphology, development, and survival of cancer cells (Treuren and Vishwanatha, 2018).

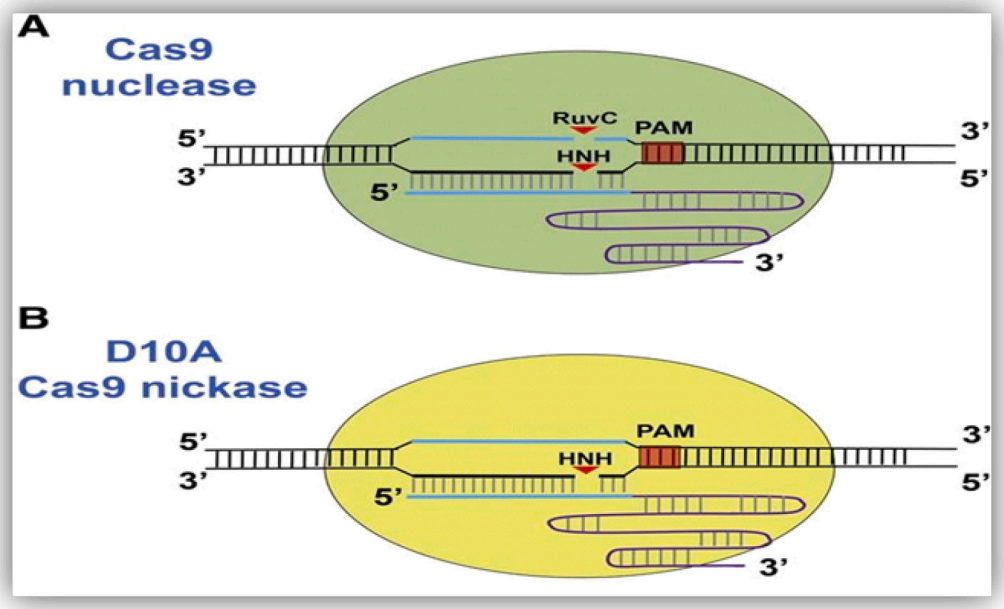

Fig. 2. Shows the difference between Cas9 nuclease and D10A Cas9 nickase (Jo et al., 2015).

On the other hand, CRISPR was exploited to target E6, an oncoprotein essential for the degradation of p53 and development of cervical cancer, resulting in a significant decrease in tumor proliferation *in vitro* and *in vivo*. Therefore, this strategy could be effective in treating cervical cancer (Yoshiba et al., 2018). Furthermore, using CRISPR/Cas9 technology, YES1 belongs to SRC family kinases, has been demonstrated to be required for lung cancer carcinogenesis; thus its knockout has given rise to inhibition of growth and metastasis of *in vivo* and *in vitro* cancer cells (Irati et al., 2019). In addition, the deletion of minichromosome maintenance 10 (MCM10) using CRISPR/Cas9 technique has indicated that MCM10 activates Akt signaling and acts as an oncogene in esophageal cancer cells; therefore, MCM10 might be a good target for treating this malignancy (Yan et al., 2018).

6. History of CRISPR

As Marcus Tullius Cicero said, "Omnium rerum principia parva sunt (the outsets of everything are small)." Huge fulfillment is impossible without small findings. A young Japanese scientist, in 1987, Yoshizumi Ishino, delineated short repeat of palindromic sequences in position of downstream *E. coli* lap gene which is currently called CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats). Ishino's research deduced: "To date, no sequences similar to those are found anywhere in procaryotes, also the biological significance of those sequences is obscure." (Ishino et al., 1987). During that period, the tools essential for additional investigations were not yet instituted. Thus, it had remained mysterious until 2000, when the Spanish scientist, Mojica, and his colleagues, accomplished comparative analysis of genomes in prokaryotes and revealed that CRISPRs exist in various species (Mojica et al., 2000).

In 2005, three groups of researchers autonomously demonstrated that short spacer sequences among CRISPRs are analogous to sequences of the viral genome (Mojica et al., 2005; Bolotin et al., 2005; Pourcel et al., 2005). In 2006, Makarova et al. suggested that CRISPR is a defense system that leads to immunological memory (Makarova et al., 2006). In 2007, Barrangou proved the former hypothesis; during their work at the yogurt manufacturer, Danisco, Barrangou's group noticed that the lactic acid bacterium losing the spacer sequences between CRISPR repeats did not display virus resistance (Barrangou et al., 2007). Those results proposed that bacteria possess an adaptive immune system that associates short sequences of foreign DNA within CRISPR positions as spacers. In August 2012, Jinek and his colleagues suggested in their study "A programmable DualRNA- guided DNA endonuclease in adaptive immunity of bacteria," that bacteria possess an adaptive immunity (Jinek et al., 2012). Subsequently, the existence of adaptive immunity in bacteria was emphasized by numerous groups that also demonstrated the fundamental mechanisms (Hatada and Horii, 2016).

The CRISPR cluster is transcribed to a precrRNA, this is treated by a transactivating CRISPR RNA (tracrRNA) which complements a fraction of pre-crRNA and develops to crRNA.

crRNA consists of two sections, a sequence that complements tracrRNA and 20-bp sequence which complements the intended DNA sequence in the virus. They concluded that crRNA base-paired to transactivating crRNA (tracrRNA) constructs two-RNA structure that guides Cas9 to make a specific dsDNA break in target (Cong et al., 2013; Mali et al., 2013). Lastly, they revealed that solely two parts, Cas9 protein and guide RNA (gRNA), composed of tracrRNA and crRNA, are needed for *in vitro* site-specific DNA cleavage. After five months of the original research publication, four researchers elucidated that CRISPR/Cas9 technology is possible to be exploited for producing knockouts in the genome of mammalian cells (Jinek et al., 2013; Cho et al., 2013). Four months later, another group was able to directly inject CRISPR/Cas9 into oocytes and produce knockout mice (Wang et al., 2013).

7. CRISPR/CAS9 is preferred over other techniques

CRISPR/Cas9 system has many benefits in terms of easiness, flexibility, and availability over two other genome editing methods. The first and most important distinction among them is that CRISPR method depends on the identification of RNA-DNA instead of the protein-DNA binding process (Jinek et al., 2012; Liu et al., 2015). For such a purpose, creating a modified CRISPR/Cas9 complex is quite feasible and simpler by merely modifying the gRNA sequence rather than designing a new protein. The objective sequence must be directly PAM upstream sequence since the latter is necessary for Cas9 to recognize the target. That short sequence arises almost once in every eight base pairs in the genome, allowing designing multiple gRNAs for a single particular target gene (Lee et al., 2016).

ZFN is the earliest and also most low-efficiency technique and more costly than any other gene-editing technologies. The principal issue is the need to recode large segments for each new target location. TALEN and CRISPR have a great target location specificity, enabling researchers to make reliable genetic modifications. This specificity is achieved by CRISPR *via* the sgRNA (Jinek et al., 2012). CRISPR is famous for its ability to modify high-frequency chromosome targets. CRISPR/Cas9 indel formation levels were recorded (more than 70%). TALEN is also capable of changing chromosomes with a significant efficacy rate, but indel production is less than CRISPR/Cas9 (33%) (Lee et al., 2016; Park et al., 2014). TALEN is methylation-sensitive, yet CRISPR-Cas9 is not methylation-sensitive (Khan et al., 2020).

8. Expectations and difficulties of CRISPR/Cas9

CRISPR/Cas9 technology displayed positive conclusions in preclinical research. It is a favorable method that allowed scientists to manipulate genes at a single bp resolution in a reasonably effective method. Preclinical researches have resulted, by using CRISPR-based technique, in identifying many possible therapeutic targets. That has gave hope for the curative applications of this technique in treating cancer. In the clinical setting, however, there are still some concerns about its application. Genes having oncogenic activity since knock-out of genes can be more appropriate in contrast to gene knock-in utilizing CRISPR/Cas9. Moreover, oncogenes are commonly over-expressed in elementary tumors and also more modifiable for suppression by pharmaceuticals. Though heterogeneous genetic of malignant-tumors, the bulk of tumor is primarily the product of the over-growth of single or double dominant clones (Ferronika et al., 2017) created in certain genes by driver mutations. sgRNAs can recognize the mutant and-wild-type allele in tumors, minimizing the effects of off-target and increasing specificity (Romero et al., 2017).

Hence, mutations hotspot in genes like BAP1,EGFR, BRAF, KRAS, BRCA1, and BRCA2 are possible to be utilized as a curative strategy in a group of patients. This approach is beneficial as normal cells, not carrying the mutant alleles, will not be targeted and therefore stay intact. Additionally, oncogene mutations, including KRAS are signals for poor prognosis and/or drug resistance in a subset of NSCLC patients (Matikas et al., 2017).

In contrast, CRISPR/Cas9 has the ability to target a particular sequence in the genome with high specificity. Thus, exchanging mutant versions of KRAS with wild-type (in p. G12V and p. G12D) alleles can enhance the therapeutic response in cancers based on KRAS. Over the last few years, a group of CRISPR-related nucleases has been found out, which can significantly improve gene editing. Despite that, some serious problems, including off-target effects, continuing Cas9 activity, poor efficacy of existing delivery approach (Fu et al., 2013a), minimum effectiveness of CRISPR/Cas9-based gene knock-in (DeWitt et al., 2016), pre-existing adaptive immune system, and unregulated repair of DNA still have to be resolved before CRISPR/Cas9 reaches the clinic (Fu et al., 2013a).

Moreover, new research has revealed that p53 is able to reduce the

effectiveness of CRISPR/Cas9 genome editing (Haapaniemi et al., 2018; Ihry et al., 2018). Depending on those findings, CRISPR/Cas9 is inclined to target intact p53 cells and leave behind cells with p53 deficiency, that can be cancerous. The assessment of p53 proteins before and after genome editing is therefore important for the care of patients. In spite of that, p53 activation, triggered by CRISPR, seems to occur solely with HDR gene editing. Base editors are not likely to induce p53 and are therefore safer than CRISPR/Cas9 technology in this regard (Ray et al., 2010a). Selecting the correct target is an essential step in creating a modern therapeutic method. The selected protein and/or molecule could not be a suitable target depending solely on its elevated expression in elementary tissues of tumor. Meanwhile, a wide *in vivo* and *in vitro* functional search are needed before conducting a clinical experiment. For instance, in breast cancer, FOXC, a transcription factor, is known to be a prognostic biomarker and was proposed as a curative target (Liu et al., 2019).

However, a new survey by Mott and his colleagues found no distinction between parental tumor cells and FOXC1 in the size and metastasis of tumors *in vivo* (Han et al., 2017). Likewise, new thought-provoking research found that maternal embryonic leucine zipper kinase (MELK) is not a target for cancer, while several continual clinical experiments are attempting to repress MELK for cancer treatment (Mott et al., 2018). Those findings emphasize the significance of *in vivo* experiments using CRISPR/Cas9 in biomarker validation and curative targets and their thoroughness. In general, four steps are existed to get a protein or molecule from the bench to the clinic as a therapeutic strategy, which is (I) detection of main molecules in various diseases; (II) validation of these molecules through *in vitro* and *in vivo* experiments; (III) advancement of an effective strategy for inhibiting the particular molecule and (IV) active stage I–III clinical experiments. If one of those steps does not succeed, modern therapeutic strategies cannot reach the clinics (Huang et al., 2017).

Another significant challenge that needs to be tackled is the decreased rate of all alleles knock- out in tumor cells, which is due to the elevated level of aneuploidy in tumor cells, which might lead to an unexpected result (Ray et al., 2010b). Hence, applying multiple gRNAs to a particular gene could enhance the probability of all alleles knock-out in tumor cells. This approach, however, could result in a subpopulation of cells having active alleles arising from an in-frame repair of double-stranded breaks at the intended position caused by different gRNAs. However, gRNAs differ in the level of efficacy; some have higher efficiency compared to others. One resolution is to accurately engineer and evaluate every gRNA for certain forms of cancer cells *in vivo* and *in vitro* and then select the most appropriate combinations. This will decrease gRNAs number and increase the efficacy of the method. Interestingly, the space among various gRNAs could also affect the effectiveness of the knockouts. Multiple carefully designed adjacent gRNAs have been shown to increase the probability of creating cells with completed gene knock-out (Gao et al., 2019).

Additionally, HDR is activated in phases S and G2/M; however, more double-stranded break repairs exist in phases G0/G1. Therefore, the use of cytostatic medications to introduce arrest cell cycle could control the repair system in a specific range (Haapaniemi et al., 2018; Ihry et al., 2018). Furthermore, new versions of Cas9, including Cpf1, can create staggered DSBs, which could lead to more precise and accurate repairs (Zetsche et al., 2015).

One of the major obstacles in applying CRISPR/Cas9 as a curative strategy in cancer treatment is the efficacy of the transmission techniques to cancer cells. More precise and effective vectors for delivery have to be designed to accomplish adequate transduction and transgene expression levels. Conventional approaches for viral delivery, like AdV, LV, and AAV, are now insufficient to achieve clinical delivery demands, particularly when aimed at cancer cells. Thus, it is more realistic to use CRISPR/Cas9 to develop the treatment strategies presently available (Liu et al., 2016). For example, CRISPR/Cas9-based engineered universal CAR-T cells could improve the antitumor effectiveness (Schukken and Foijer, 2018). Furthermore, CRISPR/Cas9 could be used to elucidate drug-resistant cancer cells and increase therapeutic sensitivity by removing and/or altering genes linked to resistance (Zuo et al., 2017). Lastly, using CRISPR/Cas9 to arm oncolytic viruses can be a new approach for cancer treatment (Phelps et al., 2018).

Another appealing use of CRISPR/Cas9 is to improve the immunotherapies associated with apoptosis protein 1 (PD-1). CRISPR-mediated PD-1 inhibition in T cells substantially represses PD-1 expression and stimulates *in vitro* cell immune response (Ray et al., 2010c). A continual phase I clinical study in China is utilizing CRISPR-designed T cells to evaluate the integrity and efficacy of that treatment depending on immunotherapy in metastatic NSCLC patients (Danner et al., 2017). In that system, peripheral T cells are extracted from patients, CRISPR/Cas9-mediated PD-1 knock-out is performed, and cells are reinfused into patients. The designed T cells resist inhibition by PD-L1-positive cancer cells and therefore implement their antitumor performance (Su et al., 2016). That form of combination treatment, a combination of CRISPR/Cas9 with CAR-T- or PD-1-related studies, in particular, might improve the results of clinical therapy for cancer in the future (Liu et al., 2019).

9. DNA repair system

When the breaks in double-stranded DNA being made by nucleases, cellular DNA repair enzymes are activated. Two repair systems can be used for repairing DNA breaks, non-homologous end joining (NHEJ), and homology-directed repair (HDR) (Ciccia and Elledge, 2010) (Fig. 3).

10. Non-homologous end joining

NHEJ takes place when repairing or ligating the damaged sides of DNA in the absence of the homologous template. For this reason, NHEJ can lead to small deletions or insertions of nucleotides in the damaged loci; thus it is usually more likely to cause errors. Even though the gene product, in the end, can be unaffected by small insertions or deletions, they could bring about mutations that subsequently alter the gene expression (Phelps et al., 2018). As NHEJ requires no homologous template to repair DNA, it could exist at any phase in the cycle of the cell, as well, it happens faster compared with the other DNA repair methods (Su et al., 2016). Regarding clinical diseases, NHEJ's natural process was mainly exploited to deactivate genes (Cyranoski, 2016).

11. Homology-directed repair

HDR differs from NHEJ since it ligates the double-stranded DNA damages using a homologous template. HDR is activated during the cell cycle's S and G2 phases and is ideally suited for cells rapidly dividing. Generally, having used a homologous sequence, this form of DNA repair has less probability to cause errors (Chen et al., 2018; Mao et al., 2008). From a clinical viewpoint, HDR is favorable for restoring mutations in genes or for integrating genes for therapeutic purposes (Cyranoski, 2016).

12. Delivery of gene editing tools to the cells

12.1. Delivery with non-viral vectors

Transfection reagents are appropriate for delivering gene-editing techniques *in vitro* to many types of cells. Therefore, *in vivo* transmission of gene-editing techniques is primarily discussed. Hu et al. 2015 (Hu et al., 2015) utilized the TurboFect Transfection Reagent for *in vivo* delivery of TALEN plasmids into K14-HPV16 mike's vagina. The researchers found that TALENs occurred primarily in aimed organs (cervix and vagina) with minimal toxicity to the entire organism. In addition, the transmission through the vagina decreased the first- pass hepatic metabolism and retained the high quantities of endonuclease in the

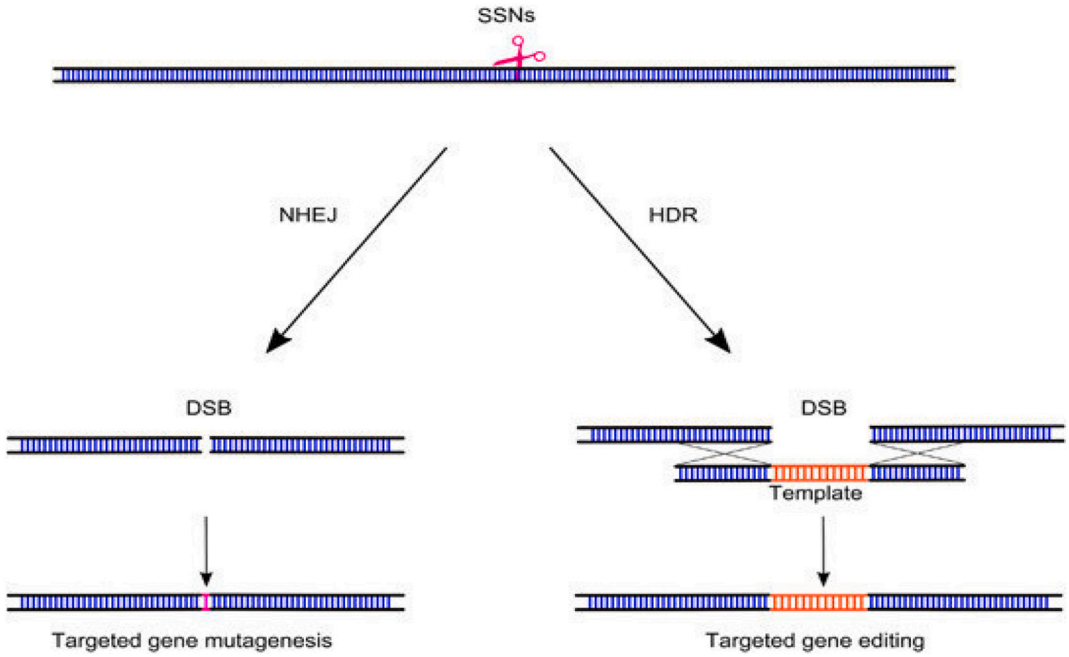

Fig. 3. Shows the mechanism of DNA repair systems, HDR, and NHEJ (Eid and Mahfouz, 2016).

intended organs. Also, the transfection through the vagina is ideal for medications not suited for systemic application. Furthermore, Hu et al. verified the efficiency of TALEN transmission in the treatment of cervical cancers in K14-HPV16 mice. As opposed to TALENs, it remains to be investigated whether ZFN and CRISPR/Cas9 perform equivalent effects or even improved performance. Additionally, certain improvements to combat cervical malignancies should be made before clinical application. For example, to sustain the long term exposure to the female vagina, the endonucleases should be in the sort of suppositories, gel, or cream. Often to be remembered are menstruation periods and the vaginal pH (Hu et al., 2015), dosage, and expense also need to be examined carefully.

However, intravenous distribution of drugs is commonly utilized. Due to the metabolization of most medications in the liver, gene editing tools could be transferred intravenously to treat HBV diseases. Systemic transmission, however, can also harm the body. Scientists would therefore pick a tissue-specific promoter so that they can ensure the expression of endonuclease in various organs (Stone et al., 2016). The alternative to systemic delivery is the hydrodynamic injection (Xue et al., 2014).

Yin and his work team 2014 (Yin et al., 2014) documented using CRISPR/Cas9 to correct Fah mutations in a mouse model with human inherited tyrosinemia. The researchers suspended the plasmid of CRISPR/Cas9 and ssDNA oligo donor in 2 mL of saline and then introduced that suspension for more than 5–7 s through the mouse tail vein. The team then identified the Fah mutation which had been corrected in 1/250 hepatocytes. Despite this, injecting a high amount of liquid in a short period can lead to high pressure in the pulmonary artery and loss of kidney function. Hydrodynamic injection therefore cannot be exploited for clinical applications.

Recently, Gao et al. 2018 (Gao et al., 2018) have used cationic lipids for transmitting Cas9 ribonucleoproteins, where sgRNA has been engineered to target transmembrane channel-like gene family 1 (Tmc1) gene in cells of hair to ameliorate autosomal dominant hearing loss. The insertion of nano compounds immediately into the cochlea has treated neonatal Tmc1Bth/+ mice. Meanwhile, decreased thresholds of aural brainstem response and improved response to auditory startle were noticed in experimental groups.

Moreover, gene editing techniques are possible to be distributed to specific organs through other means. In the future, viruses that infect lung, like Middle East respiratory coronavirus syndrome (MERS-CoV) or severe acute respiratory coronavirus syndrome (SARS-CoV) could be cured by inhalation of aerosols which contain tools for gene editing. Intraperitoneally, the delivery of retro-orbital, intra-cranial, and rectal drugs are all mechanisms for local delivery and can be utilized to transmit regional gene-editing techniques (Yu et al., 2018).

12.2. Delivery with viral vectors

Viral vectors have been commonly exploited as transmission tools. Adeno-associated virus (AAV) and lentivirus were utilized for delivering gene-editing techniques; however, they still inadequate. AAV, for instance, is able to package solely plasmids shorter than 4.2 kb (Gaj et al., 2013). Because of their small scale, ZFNs could be supplied by AAV (Ellis et al., 2013). TALENs or CRISPR/Cas9 however excel AAV's packaging ability. The two monomers of TALENs need therefore to be supplied individually *via* two AAV vectors (Gaj et al., 2013).

As for CRISPR/Cas9, AAV can be used to deliver a smaller Cas9 produced by *Staphylococcus aureus* named saCas9 (Ran et al., 2015). Lentivirus has a greater capacity of delivering than AAV; thus, TALENs or CRISPR/Cas9 could be transmitted employing lentiviral vectors. Lentivirus delivery also offers high transduction efficiency. Lentiviruses may be incorporated into intended cells to preserve the constant expression of gene editing techniques to avoid the resurgence of the virus. Viruses, however, often appear to insert into the genome of host and lead to genomic changeability. Integrase-deficient lentiviral vectors (IDLVs) are transiently expressed and cannot insert into the genetic material (Ortinski et al., 2017; Holkers et al., 2013).

Researchers can utilize IDLVs for delivering ZFNs or CRISPR/Cas9 (Ortinski et al., 2017; Lombardo et al., 2007). TALENs are however challenging to be delivered using IDLVs (Gaj et al., 2013; Holkers et al., 2013). Thus far, no ideal strategy of distribution has been sufficient for all situations. Delivery techniques have to be modified relying on the intended organs and the sort of gene-editing technique that is utilized. Researchers have to analyze the delivery strategies and select the most appropriate one (Kim and Kim, 2014).

13. Off-target effects

Off-target impacts are an important issue of gene-editing technology. Imprecise repair of the breaks in double-stranded DNA (DSBs) can lead to chromosomal rearrangements. Most of the cancer etiologies are undesired chromosomal rearrangements which are originally caused by off-target impacts (Mani et al., 2005). Consequently, attempts were made to enhance the specificity and prevent the effects of off-target in genome editing technologies. Off-target impacts differ according to the tested endonucleases. However, scholars synthesized an obligatory *Fok*I heterodimer to minimize the off-target cleavage. On the occasion that the two subunits of the ZFN connect closely together and form a compulsory dimer, DNA breaks occur (Jinek et al., 2012; Jinek et al., 2013; Vanamee et al., 2001).

A ZFN nickase has also been produced to decrease off-target breaks (Liu et al., 2013). Instead of nonhomologous end-joining, Nickase stimulates the homologous recombination repair mechanism and thus reduces off-targeting. Like ZFNs, TALENs were combined in order to make FokI heterodimer enhance specificity. Since the CRISPR/Cas9 system's specificity can solely be identified by PAM sequence and short sgRNA sequence, the off-target impacts could be strong (Fu et al., 2013b; Cho et al., 2014; Fu et al., 2014).

Furthermore, researchers have applied many approaches to reduce off-targets. For example, to minimize off-targets, extra two G nucleotides addition to sgRNA sequence at 5′ end (Frock et al., 2015). Short sgRNAs (17–18 nt) truncated at the 5′ end of the 20 nt sgRNA sequence lowered off-target impacts by 5000 times while keeping the high activity of on target (Wyvekens et al., 2015).

Additionally, the mutations in Cas9's HNH domain or RuvC domain produce a nickase (Fu et al., 2013b). Nickases cause only a nick in a single strand of DNA. Consequently, double nickases with sgRNAs are needed to create DSBs, and that way they improve their specificity (Tsai et al., 2014). Another way to decrease off-targets is to fuse a catalytically inactive Cas9, named dCas9, onto a *Fok*I domain (Guilinger et al., 2014a; Pattanayak et al., 2011; Guilinger et al., 2014b). As dimeric endonucleases have been responsive to the spacer between the left and right monomers, there is an optimum spacer between two monomers (Kleinstiver et al., 2016; Hegge et al., 2018).

To operate properly, binding needs 15–25 bp spacer between two monomers. This spacer enhances system specificity without decreasing the effects of on target (Guilinger et al., 2014b). Furthermore, a high-fidelity CRISPR/Cas9 nuclease (SpCas9-HF1) could minimize off-target impacts (Liu et al., 2018).

14. Conclusion

Gene editing technique has facilitated the advancement of cell screening, regulation of gene expression, epigenetic alteration, development of therapeutic drugs, efficient gene screening, as well as gene diagnosis. Even though the effects of off-target in applying genome editing technology still require more optimization, advanced gene-editing clusters, and much more specific nanostructured delivery tools which increased efficacy and decreased toxicity during the transmission mechanism, getting gene-editing technique clinically affordable. With further investigations of this strategy and the collaboration of the international scientific group, it is logical to conclude that the gene-editing technique eventually has the opportunity to illuminate the underlying causes of the emergence and progression of diseases, subsequently offering novel treatments and ultimately encouraging the advancement of biological sciences.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

Barrangou, R., 2014. Cas9 targeting and the CRISPR revolution. Science 344 (6185), 707–708.

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D.A., Horvath, P., 2007. CRISPR provides acquired resistance against viruses in prokaryotes. Science 315 (5819), 1709–1712.

Bogdanove, A.J., Voytas, D.F., 2011. TAL effectors: customizable proteins for DNA targeting. Science 333 (6051), 1843–1846.

Bolotin, A., Quinquis, B., Sorokin, A., Ehrlich, S.D., 2005. Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. Microbiology 151 (8), 2551–2561.

Cao, K., Jiang, W., Cao, P., Zou, Q., Xiao, S., Zhou, J., Huang, C., 2014. Talen-mediated girdin knockout downregulates cell proliferation, migration and invasion in human esophageal carcinoma ECA109 cells. Mol. Med. Rep. 10 (2), 848–854.

Carroll, D., 2011. Genome engineering with zinc-finger nucleases. Genetics 188 (4), 773–782.

Chang, Y., Yu, L., Yu, I., Jhuang, Y., Huang, W., Yang, C., Jeng, Y., 2018. Deletion of cadherin-17 enhances intestinal permeability and susceptibility to intestinal tumour formation. Pricision Medical Sciences 246 (3), 289–299.

Chen, Y., Hu, W., Lu, Y., Jiang, S., Li, C., Chen, J., Tao, D., Liu, Y., Yang, Y., Ma, Y., 2014. A TALEN-based specific transcript knock-down of PIWIL2 suppresses cell growth in HepG2 tumor cell. Cell Prolif. 47 (5), 448–456.

Chen, P., Liting, Y., You, L., 2018. Applications of CRISPR-Cas9 technology in translational research on solid-tumor cancers. The CRISPR Journal 1 (1), 47–54.

Cho, S.W., Kim, S., Kim, J.M., Kim, J.S., 2013. Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. Nat. Biotechnol. 31 (3), 230–232.

Cho, S.W., Kim, S., Kim, Y., Kweon, J., Kim, H.S., Bae, S., Kim, J.S., 2014. Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. Genome Res. 24 (1), 132–141.

Ciccia, A., Elledge, S.J., 2010. The DNA damage response: making it safe to play with knives. Mol. Cell 40 (2), 179–204.

Cong, L., Ran, F.A., Cox, D.M., Lin, S., Barretto, R.P., Habib, N., Hsu, P.D., Wu, X., Jiang, W., Marraffini, L.A., 2013. Multiplex genome engineering using CRISPR/Cas systems. Science 339 (6121), 819–823.

Cuculis, L., Schroeder, C.M., 2017. A single-molecule view of genome editing proteins: biophysical mechanisms for TALEs and CRISPR/Cas9. Annual Review of Chemical and Biomolecular Engineering 8 (1), 577–597.

Cyranoski, D., 2016. CRISPR gene-editing tested in a person for the first time. Nature 539 (7630), 479.

Danner, E., Bashir, S., Yumlu, S., Wurst, W., Wefers, B., Kühn, R., 2017. Control of gene editing by manipulation of DNA repair mechanisms. Mamm. Genome 28 (7), 262–274.

DeWitt, M.A., Magis, W., Bray, N.L., Wang, T., Berman, J.R., Urbinati, F., Heo, S.J., Mitros, T., Muñoz, T.P., Boffelli, D., 2016. Selection-free genome editing of the sickle mutation in human adult hematopoietic stem/progenitor cells. Sci. Transl. Med. 8 (360).

Doudna, J.A., Charpentier, E., 2014. The new frontier of genome engineering with CRISPR-Cas9. Science 346 (6213), 1258096.

Eid, A., Mahfouz, M.M., 2016. Genome editing: the road of CRISPR/Cas9 from bench to clinic. Experimental and Molecular Medicine 48 (10), 1–11.

Eißmann, M., Gutschner, T., Hämmerle, M., Günther, S., Herger, M., Groß, M., Schirmacher, P., Rippe, K., Braun, T., Zörnig, M., 2012. Loss of the abundant nuclear non-coding RNA MALAT1 is compatible with life and development. RNA Biol. 9 (8), 1076–1087.

Ellis, B.L., Hirsch, M.L., Porter, S.N., Samulski, R.J., Porteus, M.H., 2013. Zinc-finger nuclease-mediated gene correction using single AAV vector transduction and enhancement by Food and Drug Administration-approved drugs. Gene Ther. 20 (1), 35–42.

Erickson, R.P., 2010. Somatic gene mutation and human disease other than cancer: an update. Mutation Research-Reviews in Mutation Research 705 (2), 96–106.

Fagerlund, R.D., Staals, R.H., Fineran, P.C., 2015. The Cpf1 CRISPR-Cas protein expands genome-editing tools. Genome Biol. 16 (1), 251.

Ferronika, P., Bos, H., Taudt, A., Spierings, D.C., Saber, A., Hiltermann, T.J., Kok, K., Porubsky, D., Wekken, A.J., Timens, W., 2017. Copy number alterations assessed at the single-cell level revealed mono- and polyclonal seeding patterns of distant metastasis in a small-cell lung cancer patient. Ann. Oncol. 28 (7), 1668–1670.

Frock, R.L., Hu, J., Meyers, R.M., Ho, Y.J., Kii, E., Alt, F.W., 2015. Genome-wide detection of DNA double-stranded breaks induced by engineered nucleases. Nat. Biotechnol. 33 (2), 179–186.

Fu, Y., Foden, J.A., Khayter, C., Maeder, M.L., Reyon, D., Joung, J.K., Sander, J.D., 2013a. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. Nat. Biotechnol. 31 (9), 822–826.

Fu, Y., Foden, J.A., Khayter, C., Maeder, M.L., Reyon, Joung, J.K., Sander, J.D., 2013b. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. Nat. Biotechnol. 31 (9), 822–826.

Fu, Y., Sander, J.D., Reyon, D., Cascio, V.M., Joung, J.K., 2014. Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. Nat. Biotechnol. 32 (3), 279–284.

Furutani, E., Shimamura, A., 2017. Germline genetic predisposition to hematologic malignancy. J. Clin. Oncol. 35 (9), 1018–1028.

Gaj, T., Gersbach, C.A., Barbas, C.F., 2013. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. 31 (7), 397–405.

Gao, X., Tao, Y., Lamas, V., Huang, M., Yeh, W.H., Pan, B., Hu, Y.J., Hu, J.H., Thompson, D.B., Shu, Y., 2018. Treatment of autosomal dominant hearing loss by in vivo delivery of genome editing agents. Nature 553 (7687), 217–221.

Gao, Q., Dong, X., Xu, Q., Zhu, L., Wang, F., Hou, Y., Chao, C., 2019. Therapeutic potential of CRISPR/Cas9 gene editing in engineered T-cell therapy. Cancer Medicine 8 (9), 4254–4264.
Gasiunas, G., Barrangou, R., Horvath, P., Siksnys, V., 2012. Cas9–crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc. Natl. Acad. Sci. U. S. A. 109 (39), 15539–15540.
Guilinger, J.P., Thompson, D.B., Liu, D.R., 2014a. Fusion of catalytically inactive Cas9 to FokI nuclease improves the specificity of genome modification. Nat. Biotechnol. 32 (6), 577–582.
Guilinger, J.P., Pattanayak, V., Reyon, D., Tsai, S.Q., Sander, J.D., Joung, J.K., Liu, D.R., 2014b. Broad specificity profiling of TALENs results in engineered nucleases with improved DNA-cleavage specificity. Nat. Methods 11 (4), 429–435.
Haapaniemi, E., Botla, S., Persson, J., Schmierer, B., Taipale, J., 2018. CRISPR–Cas9 genome editing induces a P53-mediated DNA damage response. Nat. Med. 24 (7), 1.
Han, B., Bhowmick, N., Qu, Y., Chung, S., Giuliano, A.E., Cui, X., 2017. FOXC1: an emerging marker and therapeutic target for cancer. Oncogene 36 (28), 3957–3963.
Hatada, I., Horii, T., 2016. Genome editing: a breakthrough in life science and medicine. Endocr. J. 63 (2), 105–110.
Hegge, B., Sjøttem, E., Mikkola, I., 2018. Generation of a PAX6 knockout glioblastoma cell line with changes in cell cycle distribution and sensitivity to oxidative stress. BMC Cancer 18 (1), 496.
Hilton, I.B., Gersbach, C.A., 2015. Enabling functional genomics with genome engineering. Genome Res. 25 (10), 1442–1455.
Holkers, M., Maggio, I., Liu, J., Janssen, J.M., Miselli, F., Mussolino, C., Recchia, A., Cathomen, T., Gonçalves, M., 2013. Differential integrity of TALE nuclease genes following adenoviral and lentiviral vector gene transfer into human cells. Nucleic Acids Res. 41 (5), 1–14.
Hoyert, D.L., 2012. 75 Years of Mortality in the United States, 1935–2010. NCHS Data Brief, 88 (88), pp. 1–8.
Hu, Z., Ding, W., Zhu, D., Yu, L., Jiang, X., Wang, X., Zhang, C., Wang, L., Ji, T., Liu, D., 2015. TALEN-mediated targeting of HPV oncogenes ameliorates HPV-related cervical malignancy. J. Clin. Investig. 125 (1), 425–436.
Huang, H.T., Seo, H.S., Zhang, T., Wang, Y., Jiang, B., Li, Q., Buckley, D.L., Nabet, B., Roberts, J.M., Paulk, J., 2017. MELK is not necessary for the proliferation of basal-like breast cancer cells. ELife. 6, 1–29.
Ihry, R.J., Worringer, K.A., Salick, M.R., Frias, E., Ho, D., Theriault, K., Kommineni, S., Chen, J., Sondey, M., Ye, C., 2018. P53 inhibits CRISPR–Cas9 engineering in human pluripotent stem cells. Nat. Med. 24 (7), 939–946.
Irati, G., Pajares, M.J., Prado, F.H., Ajona, D., Bértolo, C., Sainz, C., Lavín, A., Remírez, A. B., Valencia, K., Moreno, H., et al., 2019. YES1 drives lung cancer growth and progression and predicts sensitivity to dasatinib. Am. J. Respir. Crit. Care Med. 200 (7), 888–899.
Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., Nakata, A., 1987. Nucleotide sequence of the Iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. J. Bacteriol. 169 (12), 5429–5433.
Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., Charpentier, E., 2012. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337 (6096), 816–821.
Jinek, M., East, A., Cheng, A., Lin, S., Ma, E., Doudna, J., 2013. RNA-programmed genome editing in human cells. ELife 2.
Jo, Yl, Suresh, B., Kim, H., Ramakrishna, S., 2015. CRISPR/Cas9 system as an innovative genetic engineering tool: enhancements in sequence specificity and delivery methods. Biochim. Biophys. Acta 1856 (2), 234–243.
Joung, J.K., Sander, J.D., 2013. TALENs: a widely applicable technology for targeted genome editing. Nat. Rev. Mol. Cell Biol. 14 (1), 49–55.
Khan, M.N., Islam, K., Ashraf, A., Barman, N.C., 2020. A review on genome editing by CRISPR-CAS9 technique for cancer treatment. WCRJ. 7, e1510.
Kim, H., Kim, J.S., 2014. A guide to genome engineering with programmable nucleases. Nat. Rev. Genet. 15 (5), 321–334.
Kleinstiver, B.P., Pattanayak, V., Prew, M.S., Tsai, S.Q., Nguyen, N.T., Zheng, Z., Joung, J.K., 2016. High-fidelity CRISPR–Cas9 nucleases with no detectable genome-wide off-target effects. Nature 529 (7587), 490–495.
Lee, J.W., Ryu, Y.K., Ji, Y.H., Kang, J.H., Moon, E.Y., 2015. Hypoxia/reoxygenation-experienced cancer cell migration and metastasis are regulated by Rap1- and Rac1-GTPase activation via the expression of thymosin beta-4. Oncotarget 6 (12), 9820–9833.
Lee, C.M., Cradick, T.J., Bao, G., 2016. The Neisseria meningitidis CRISPR-Cas9 system enables specific genome editing in mammalian cells. Mol. Ther. 24 (3), 645–654.
Li, H.W., Yang, X.M., Tang, J., Wang, S.J., Chen, Z.N., Jiang, J.L., 2015. Effects of HAb18G/CD147 knockout on hepatocellular carcinoma cells in vitro using a novel zinc-finger nuclease-targeted gene knockout approach. Cell Biochem. Biophys. 71 (2), 881–890.
Liu, X., Wang, Y., Guo, W., Chang, B., Liu, J., Guo, Z., Quan, F., Zhang, Y., 2013. Zinc-finger nickase-mediated insertion of the lysostaphin gene into the beta-casein locus in cloned cows. Nat. Commun. 4 (1), 2565.
Liu, B., Xu, H., Miao, J., Zhang, A., Kou, X., Li, W., Zhou, L., Xie, H.G., Sirois, P., Li, K., 2015. CRISPR/Cas: a faster and more efficient gene editing system. J. Nanosci. Nanotechnol. 15 (3), 1946–1959.
Liu, T., Li, Z., Zhang, Q., De, A.K., Lozano-Calderon, S., Choy, E., Hornicek, F.J., Duan, Z., 2016. Targeting ABCB1 (MDR1) in multi-drug resistant osteosarcoma cells using the CRISPR-Cas9 system to reverse drug resistance. Oncotarget 7 (50), 83502–83513.
Liu, J., Sareddy, J.R., Zhou, M., Viswanadhapalli, S., Li, X., Lai, Z., Tekmal, R.R., Brenner, A.J., Vadlamudi, R.K., 2018. Differential effects of estrogen receptor beta isoforms on glioblastoma progression. Cancer Res. 78 (12), 3176–3189.
Liu, B., Saber, A., Haisma, H.J., 2019. CRISPR/Cas9: a powerful tool for identification of new targets for cancer treatment. Drug Discov. Today 24 (4), 955–970.
Lombardo, A., Genovese, P., Beausejour, C.M., Colleoni, S., Lee, Y.L., Kim, K.A., Ando, D., Urnov, F.D., Galli, C., Gregory, P.D., 2007. Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery. Nat. Biotechnol. 25 (11), 1298–1306.
Makarova, K.S., Grishin, N.V., Shabalina, S.A., Wolf, Y.I., Koonin, E.V., 2006. A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action. Biol. Direct 1 (1), 7.
Mali, P., Yang, L., Esvelt, K.M., Aach, J., Guell, M., DiCarlo, J.E., Norville, J.E., Church, G.M., 2013. RNA-guided human genome engineering via Cas9. Science 339 (6121), 823–826.
Mani, M., Smith, J., Kandavelou, K., Berg, J.M., Chandrasegaran, S., 2005. Binding of two zinc finger nuclease monomers to two specific sites is required for effective double-strand DNA cleavage. Biochem. Biophys. Res. Commun. 334 (4), 1191–1197.
Mao, Z., Bozzella, M., Seluanov, A., Gorbunova, V., 2008. DNA repair by nonhomologous end joining and homologous recombination during cell cycle in human cells. Cell Cycle 7 (18), 2902–2906.
Marchiq, I., Floch, R.L., Roux, D., Simon, M.P., Pouyssegur, J., 2015. Genetic disruption of lactate/H+ symporters (MCTs) and their subunit CD147/BASIGIN sensitizes glycolytic tumor cells to phenformin. Cancer Res. 75 (1), 171–180.
Matikas, A., Mistriotis, D., Georgoulias, V., Kotsakis, A., 2017. Targeting KRAS mutated non-small cell lung cancer: a history of failures and a future of hope for a diverse entity. Critical Reviews in Oncology Hematology 110, 1–12.
Miller, J.C., Holmes, M.C., Wang, J., Guschin, D.Y., Lee, Y., Rupniewski, I., Beausejour, C.M., Waite, A.J., Wang, N.S., Kim, K.A., 2007. An improved zinc-finger nuclease architecture for highly specific genome editing. Nat. Biotechnol. 25 (7), 778–785.
Mojica, F.J., Villaseñor, C.D., Soria, E., Juez, G., 2000. Biological significance of a family of regularly spaced repeats in the genomes of archaea, bacteria and mitochondria. Mol. Microbiol. 36 (1), 244–246.
Mojica, F.J., Villaseñor, C.D., Martínez, J.G., Soria, E., 2005. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. J. Mol. Evol. 60 (2), 174–182.
Moore, F.E., Reyon, D., Sander, J.D., Martinez, S.A., Blackburn, J.S., Khayter, C., Ramirez, C.L., Joung, J.K., Langenau, D.M., 2012. Improved somatic mutagenesis in zebrafish using transcription activator-like effector nucleases (TALENs). PLoS One 7 (5).
Mott, L., Su, K., Pack, D.W., 2018. Evaluation of FOXC1 as a therapeutic target for basal-like breast cancer. Cancer Gene Ther. 25 (3), 84–91.
Mussolino, C., Alzubi, J., Pennucci, V., Turchiano, G., Cathomen, T., 2017. Genome and epigenome editing to treat disorders of the hematopoietic system. Hum. Gene Ther. 28 (11), 1105–1115.
Ortinski, P.I., O'Donovan, B., Dong, X., Kantor, B., 2017. Integrase-deficient lentiviral vector as an all-in-one platform for highly efficient CRISPR/Cas9-mediated gene editing. Molecular Therapy. Methods & Clinical Development. 5, 153–164.
Palti, Y., 1962. Stimulation of muscles and nerves by means of externally applied electrodes. Bulletin of the Research Council of Israel. Section E: Experimental Medicine 10, 54–56.
Park, C.Y., Kim, J., Kweon, J., Son, J.S., Lee, J.S., Yoo, J.E., Cho, S.R., Kim, J.H., Kim, J. S., Kim, D.W., 2014. Targeted inversion and reversion of the blood coagulation factor 8 gene in human IPS cells using TALENs. Proc. Natl. Acad. Sci. U. S. A. 111 (25), 9253–9258.
Pattanayak, V., Ramirez, C.L., Joung, J.K., Liu, D.R., 2011. Revealing off-target cleavage specificities of zinc-finger nucleases by in vitro selection. Nat. Methods 8 (9), 765–770.
Phelps, M.P., Yang, H., Patel, S., Rahman, M.M., McFadden, G., Chen, E., 2018. Oncolytic virus-mediated RAS targeting in rhabdomyosarcoma. Molecular Therapy – Oncolytics 11, 52–61.
Pourcel, C., Salvignol, G., Vergnaud, G., 2005. CRISPR elements in Yersinia pestis acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. Microbiology 151 (3), 653–663.
Puria, R., Sahi, S., Nain, V., 2012. HER2+ breast cancer therapy: by CPP-ZFN mediated targeting of mTOR? Technology in Cancer Research & Treatment 11 (2), 175–180.
Qi, L.S., Larson, M.H., Gilbert, L.A., Doudna, J.A., Weissman, J.S., Arkin, A.P., Lim, W.A., 2013. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell 152 (5), 1173–1183.
Ran, F.A., Cong, L., Yan, W.X., Scott, D.A., Gootenberg, J.S., Kriz, A.J., Zetsche, B., Shalem, O., Wu, X., Makarova, K.S., 2015. In vivo genome editing using staphylococcus aureus Cas9. Nature 520 (7546), 186–191.
Ray, P.S., Wang, J., Qu, Y., Sim, M.S., Shamonki, J., Bagaria, S.P., Ye, X., Liu, B., Elashoff, D., Hoon, D.S., 2010a. FOXC1 is a potential prognostic biomarker with functional significance in basal-like breast cancer. Cancer Res. 70 (10), 3870–3876.
Ray, P.S., Wang, J., Qu, Y., Sim, M.S., Shamonki, J., Bagaria, S.P., Ye, X., Liu, B., Elashoff, D., Hoon, D.S., 2010b. FOXC1 is a potential prognostic biomarker with functional significance in basal-like breast cancer. Cancer Res. 70 (10), 3870–3876.
Ray, P.S., Wang, J., Qu, Y., Sim, M.S., Shamonki, J., Bagaria, S.P., Ye, X., Liu, B., Elashoff, D., Hoon, D.S., 2010c. FOXC1 is a potential prognostic biomarker with functional significance in basal-like breast cancer. Cancer Res. 70 (10), 3870–3876.
Ren, Y., Qiu, L., Lü, F., Ru, X., Li, S., Xiang, Y., Yu, S., Zhang, Y., 2016. TALENs-directed knockout of the full-length transcription factor Nrf1α that represses malignant behaviour of human hepatocellular carcinoma (HepG2) cells. Sci. Rep. 6 (1), 23775.
Romero, R., Sayin, V.I., Davidson, S.M., Bauer, M.R., Singh, S.X., LeBoeuf, S.E., Karakousi, T.R., Ellis, D.C., Bhutkar, A., Rivera, F.J., 2017. Keap1 loss promotes

Kras-driven lung cancer and results in dependence on glutaminolysis. Nat. Med. 23 (11), 1362–1368.
Sapranauskas, R., Gasiunas, G., Fremaux, C., Barrangou, R., Horvath, P., Siksnys, V., 2011. The Streptococcus thermophilus CRISPR/Cas system provides immunity in Escherichia coli. Nucleic Acids Res. 39 (21), 9275–9282.
Schukken, K.M., Foijer, F., 2018. CIN and aneuploidy: different concepts, different consequences. BioEssays 40 (1), 1700147.
Scott, C.T., 2005. The zinc finger nuclease monopoly. Nat. Biotechnol. 23 (8), 915–918.
Shankar, S., Prasad, D., Sanawar, R., Das, A.V., Pillai, M.R., 2017. TALEN based HPV-E7 editing triggers necrotic cell death in cervical cancer cells. Sci. Rep. 7 (1), 1–12.
del Sol, A., Balling, R., Hood, L., Galas, D., 2010. Diseases as network perturbations. Curr. Opin. Biotechnol. 21 (4), 566–571.
Stone, D., Niyonzima, N., Jerome, K.R., 2016. Genome editing and the next generation of antiviral therapy. Hum. Genet. 135 (9), 1071–1082.
Su, S., Hu, B., Shao, J., Shen, B., Du, J., Du, Y., Zhou, J., Yu, L., Zhang, L., Chen, F., 2016. CRISPR-Cas9 mediated efficient PD-1 disruption on human primary T cells from cancer patients. Sci. Rep. 6 (1), 20070.
Sun, Y., Ji, P., Chen, T., Zhou, X., Yang, D., Guo, Y., Liu, Y., Hu, L., Xia, D., Liu, Y., 2017. MIIP haploinsufficiency induces chromosomal instability and promotes tumour progression in colorectal cancer. J. Pathol. 241 (1), 67–79.
Szczepek, M., Brondani, V., Büchel, J., Serrano, L., Segal, D.J., Cathomen, T., 2007. Structure-based redesign of the dimerization interface reduces the toxicity of zinc-finger nucleases. Nat. Biotechnol. 25 (7), 786–793.
Torre, L.A., Bray, F., Siegel, R.L., Ferlay, J., Lortet-Tieulent, J., Jemal, A., 2015. Global cancer statistics, 2012. CA Cancer J. Clin. 65 (2), 87–108.
Treuren, T.V., Vishwanatha, J.K., 2018. CRISPR deletion of MIEN1 in breast cancer cells. PLoS One 13 (10).
Tsai, S.Q., Wyvekens, N., Cyd, Khayter, Foden, J.A., Thapar, V., Reyon, D., Goodwin, M. J., Aryee, M.J., Joung, J.K., 2014. Dimeric CRISPR RNA-guided FokI nucleases for highly specific genome editing. Nat. Biotechnol. 32 (6), 569–576.
Urnov, F.D., Rebar, E.J., Holmes, M.C., Zhang, H.S., Gregory, P.D., 2010. Genome editing with engineered zinc finger nucleases. Nat. Rev. Genet. 11 (9), 636–646.
Vanamee, É.S., Santagata, S., Aggarwal, A.K., 2001. FokI requires two specific DNA sites for cleavage. J. Mol. Biol. 309 (1), 69–78.
Vogelstein, B., Papadopoulos, N., Velculescu, V.E., Zhou, S., Diaz, L.A., Kinzler, K.W., 2013. Cancer genome landscapes. Science 339 (6127), 1546–1558.
Wang, H., Yang, H., Shivalila, C.S., Dawlaty, M.M., Cheng, A.W., Zhang, F., Jaenisch, R., 2013. One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell 153 (4), 910–918.
Wyvekens, N., TopkarVed, V., KhayterCyd, Keith, J., TsaiShengdar, Q., 2015. Dimeric CRISPR RNA-guided FokI-DCas9 nucleases directed by truncated GRNAs for highly specific genome editing. Hum. Gene Ther. 26 (7), 425–431.
Xue, W.B., Chen, S., Yin, H., Tammela, T., Papagiannakopoulos, T., Joshi, N.S., Cai, W., Yang, G., Bronson, R.T., Crowley, D.G., 2014. CRISPR-mediated direct mutation of cancer genes in the mouse liver. Nature 514 (7522), 380–384.
Yan, J., Du, P., Jia, X., Chang, Z., Gan, S., Xu, X., Wang, Y., Qin, Y., Kan, Q., 2018. Ablation of MCM10 using CRISPR/Cas9 restrains the growth and migration of esophageal squamous cell carcinoma cells through inhibition of Akt signaling. OncoTargets and Therapy 11, 3323–3333.
Yi, L., Li, J., 2016. CRISPR-Cas9 therapeutics in cancer: promising strategies and present challenges. Biochim. Biophys. Acta 1866 (2), 197–207.
Yin, H., Xue, W., Chen, S., Bogorad, R.L., Benedetti, E., Grompe, M., Koteliansky, V., Sharp, P.A., Jacks, T., Anderson, D.G., 2014. Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. Nat. Biotechnol. 32 (6), 551–553.
Yoshiba, T., Saga, Y., Urabe, M., Uchibor, R., Matsubara, S., Fujiwara, H., Mizukami, H., 2018. CRISPR/Cas9-mediated cervical cancer treatment targeting human papillomavirus E6. Oncol. Lett. 17 (2), 2197–2206.
Yu, L., Tian, X., Gao, C., Wu, P., Wang, L., Feng, B., Li, X., Ma, D., Wang, H., Hu, Z., 2018. Genome editing for the treatment of tumorigenic viral infections and virus-related carcinomas. Frontiers of Medicine in China 12 (5), 497–508.
Zetsche, B., Gootenberg, J.S., Abudayyeh, O.O., Slaymaker, I.M., Makarova, K.S., Essletzbichler, P., Volz, S.E., Joung, J., Oost, J.V., Regev, A., 2015. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell 163 (3), 759–771.
Zhan, T., Rindtorff, N., Betge, J., Ebert, M.P., Boutros, M., 2019. CRISPR/Cas9 for cancer research and therapy. Semin. Cancer Biol. 55, 106–119.
Zuo, E., Cai, Y.J., Li, K., Wei, Y., Wang, B.A., Sun, Y., Liu, Z., Liu, J., Hu, X., Wei, W., 2017. One-step generation of complete gene knockout mice and monkeys by CRISPR/Cas9-mediated gene editing with multiple SgRNAs. Cell Res. 27 (7), 933–945.