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Research progress of CRISPR-Cas9 gene editing technology in construction of cancer models

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Abstract. Gene editing broadly refers to a range of scientific techniques that can override a cell's intrinsic genetic code. Gene editing may, for instance, have a crucial therapeutic role in altering the expression of genes that can contribute to the development of cancer by modulating oncogenic signaling pathways. Cancer is one of the most fatal diseases around the globe. Despite advances in diagnostics and therapeutics, curative therapies have been challenging to identify. Numerous therapeutic methods have already been found and developed, such as small molecule inhibitors and gene therapies, but novel therapeutic strategies, in the form of gene editing, are required to be explored. CRISPR-Cas9 gene editing technology, combined with advancing computer and imaging capabilities has made it possible to accurately diagnose diseases, predict disease susceptibility based on individual genome, and even to modify genetic information. It has important application value in gene function research, tumor mouse model construction and cancer therapy because of its high efficiency and precision. The discovery and advancement of CRISPR-Cas9 may shed light on the future direction for cancer treatment and present a solution for curing cancer. In this review, the mechanisms of action of CRISPR-Cas9 and its delivery into cells will be explored. Both in vitro and in vivo cancer models created by CRISPR-Cas9 will be discussed and the advantages and limitations of CRISPR-Cas9 system will be examined.

Keywords: CRISPR-Cas9 system, cancer, models.

1. Introduction

CRISPR-Cas9 is a naturally occurring gene editing system, which is used by microorganisms for immune protection [1]. Clustered regularly interspaced short palindromic repeat (CRISPR) refers to short DNA sequences, known as spacers, which are extracted from a bacteriophage that has previously infected the prokaryote. The palindromic repeats ensure that pre-CRISPR RNA (crRNA) can be correctly processed into small crRNA as well as avoiding auto-immune cleavage. In the following infection, Cas protein can cause DNA cleavage by binding with phage genome. Scientists repurposes the immune defense system to target cellular gene sequences. CRISPR–Cas9-targeted fragmentation of DNA is a novel strategy for gene editing and can be used to pinpoint the changes in cancer-specific regions.

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2. CRISPR/CAS-9 genome editing

2.1. Delivery of CRISPR/Cas-9 into cells

There are three key mechanisms for inserting the CRISPR–Cas9 into a host genome which are explored in Table 1 [2-4].

Table 1. A comparison of three common viral vectors.

	Positives	Limitations
Adeno-associated virus	1) Non-immunogenic 2) No host inflammatory responses 3) Efficient entry of DNA into target cells 4) Long-term DNA persistence in target cells	1) Requires conversion to double-stranded DNA 2) Small packaging limit
Adenoviral vectors	1) Transduce non-dividing and dividing cells 2) Carry 8Hbp heterogeneous DNA 3) High levels of transgene expression	1) Highly immunogenic 2) Doesn't integrate into host genome 3) High-levels of pre-existing immunity
Lentivirus	1) Long-term gene expression 2) Both dividing and non- dividing cells can be infected	1) Has ability to replicate

2.1.1. Adeno-associated viruses (AAVs). AAV is a small unenveloped virus with a single strand of DNA. AAVs are suitable as delivery vectors for CRISPR-Cas9 as they are not pathogenic to humans since they are dependent on helper viruses for replication. Both dividing and non-dividing cells can be infected by AAVs [5].

Moreover, AAVs afford an abundance of serotypes, different serotypes can infect a variety of cells. For example, AAV9 can cross the blood-brain-barrier in humans, AAV7 can be efficiently transfected into mouse skeletal muscle, and AAV5 transduces astrocytes [6-8]. As a result, this extensive range of viral tropism of AAVs allows for tissue-specific applications, such as transducing organoids in cancer models.

However, innate immune responses and pre-existing neutralizing antibodies to AAV vectors have been observed for repeated injection. Fortunately, this issue can be resolved by applying different AAV serotypes [9, 10].

2.1.2. Adenoviruses (AdVs). In contrast to AAVs, adenoviruses (AdVs) are medium-sized, non-enveloped viruses with double-stranded DNA from the family of Adenoviridae. In a similar fashion to AAVs, AdVs have the ability to infect a broad range of mitotic and non-mitotic cells [11].

AdVs would be one of the top vector choices for delivering the CRISPR-Cas9 system as they can transport a significantly large genetic load [5]. ADVs can carry a genome size of up to 37 kb, which exceeds the size limitation for delivering the CRISPR/Cas9 system. This impressive packaging capacity ensures optimal genetic transmission, which cannot be achieved using alternative viral vectors.

One major disadvantage of the CRISPR-Cas9 is the off-target effects, which can fortunately be eliminated by the use of adenoviral vector, as it does not interfere with the host genome. Indeed, the adenovirus creates episomal DNA which lies adjacent to the host DNA.

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Moreover, the AdV structural proteins are highly adaptable, which means that researchers can create AdVs with high specific tissue tropism. Additionally, the production of Advs is economically efficient and they can be produced in large quantities at relatively low cost. This concept is best illustrated by the fact that AdVs were most widely explored during the development of mRNA vaccines for the COVID-19 pandemic [5].

2.1.3. Lentivirus. Lentivirus, which has a single strand RNA contained within a viral envelope with spike proteins covering its surface, belongs to the retrovirus genus. Contemporary recombinant lentivectors are principally derived from the human immunodeficiency virus (HIV) [12]. Moreover, the transgene can be expressed for a long time to ensure that the modifications made by the CRISPR CAS-9 system are not temporary [12]. Not akin to other retroviruses, lentiviruses can pass through the nucleus irrespective of whether they are undergoing cell division. Some cells, such as nerve cells, do not undergo mitosis in adults, so lentiviruses offer an alternative pathway (Figure 1) [13].

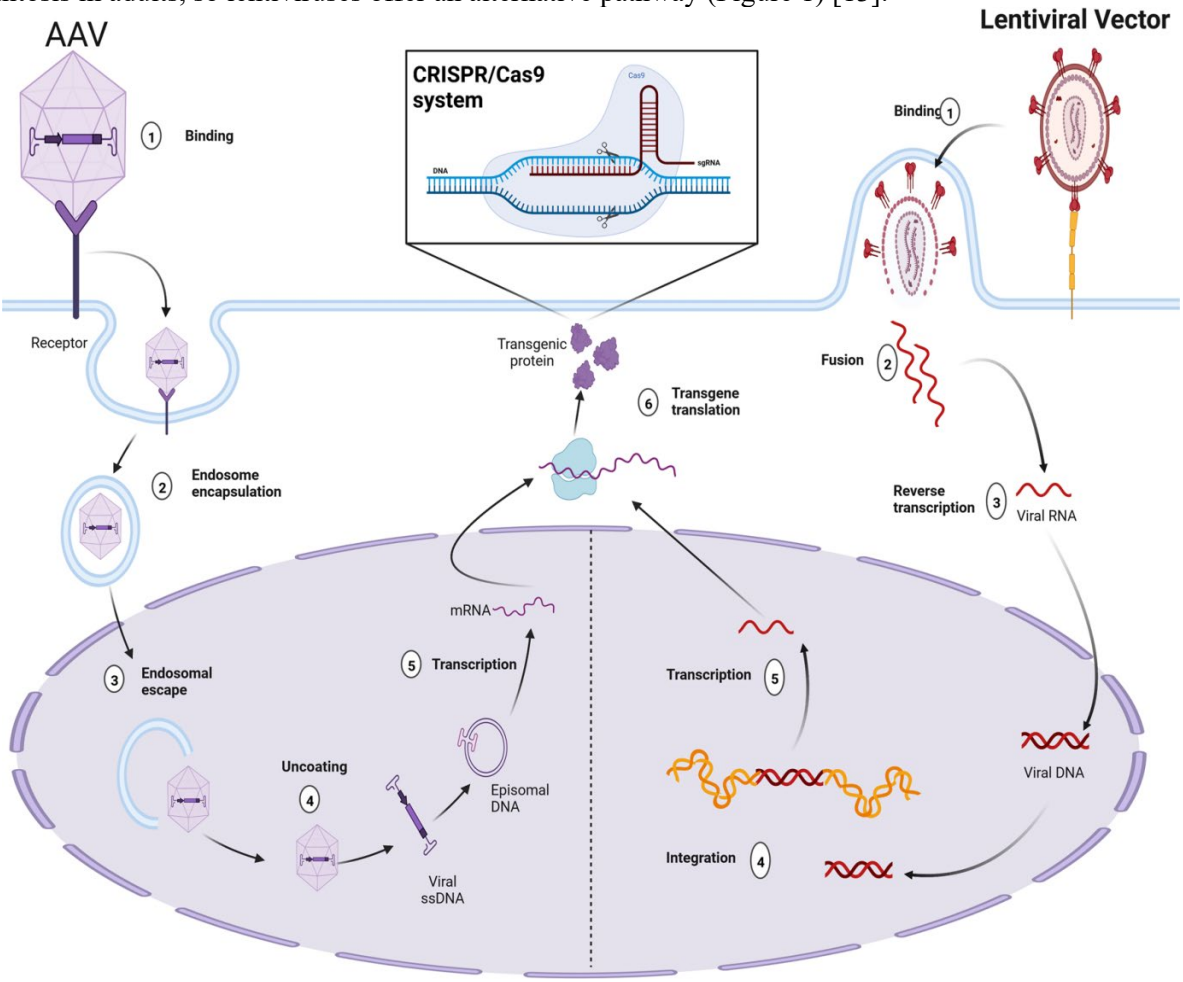

Figure 1. The mechanism of action of the adeno-associated virus and the lentiviral vectors which can transmit the CRISPR-Cas9 system [5].

2.2. Structure

In CRISPR/Cas system, two categories are stratified depending on the number of Cas endonucleases (Figure 2). Class I uses numerous calcium sulfide proteins to function, while Class II requires one calcium sulfide enzyme. CRISPR-Cas9 was grouped into the second class [5]. Cas9 is a DNA endonuclease with multiple functional regions, which cleaves the target DNA, resulting in DNA damage. Cas9 protein is separated into six domains: REC I, REC II, Bridge Helix, PAM Interacting, HNH and

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RuvC. REC I domain is associated with gRNA. The PAM-Interacting domain commences binding to target DNA by affording PAM specificity. The HNH and RuvC are nucleases which can cleave single-stranded DNA [14].

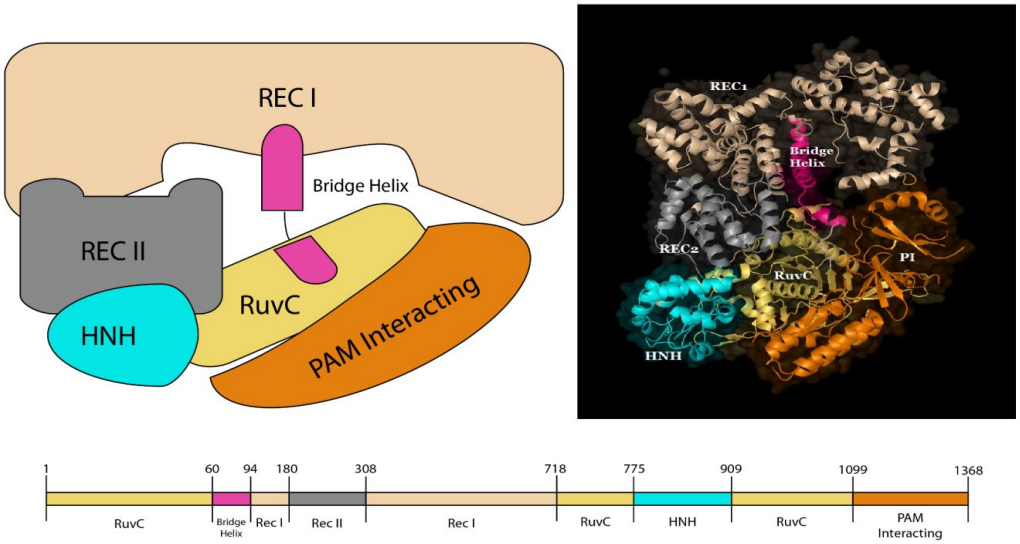

Figure 2. Cas-9 protein [14].

In genetic engineering, crRNA and tracrRNA have been replaced by a single guide RNA (sgRNA) [15]. As shown in Figure 3, Guide RNA is a T-shaped molecule, composed of a single strand of RNA which is complementary to the target DNA sequence, one tetraloop and two or three stem loops.

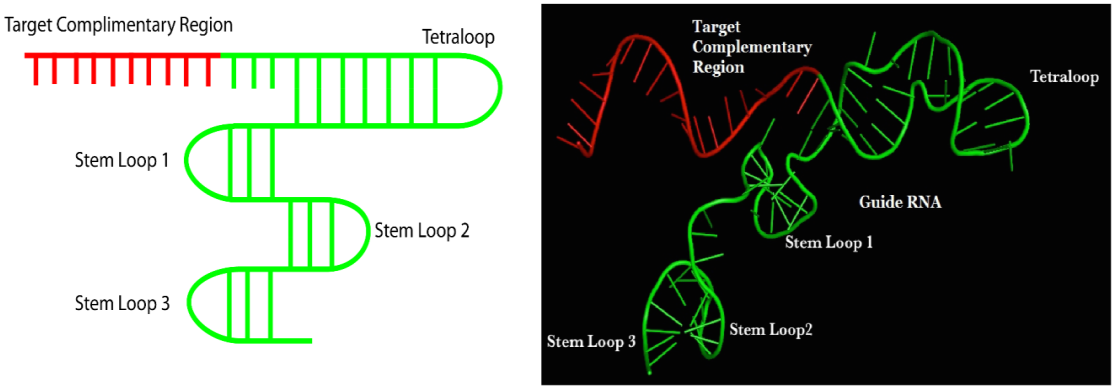

Figure 3. The structure of guide RNA [14].

2.3. Mechanism of CRISPR-Cas9

2.3.1. Recognition. CRISPR-Cas9 complex can recognize target DNA by matching with PAM. PAM is a two-or three-base sequence located one nucleotide downstream from the end of the target gene. The binding causes the DNA to unwind directly upstream of the PAM, and the target sequence will be paired with the guide RNA's target complementary region [14].

2.3.2. Cleavage. If the pairing is successful, RuvC and HNH nuclease will cut the target gene at three bases upstream of PAM (Figure 4) [15].

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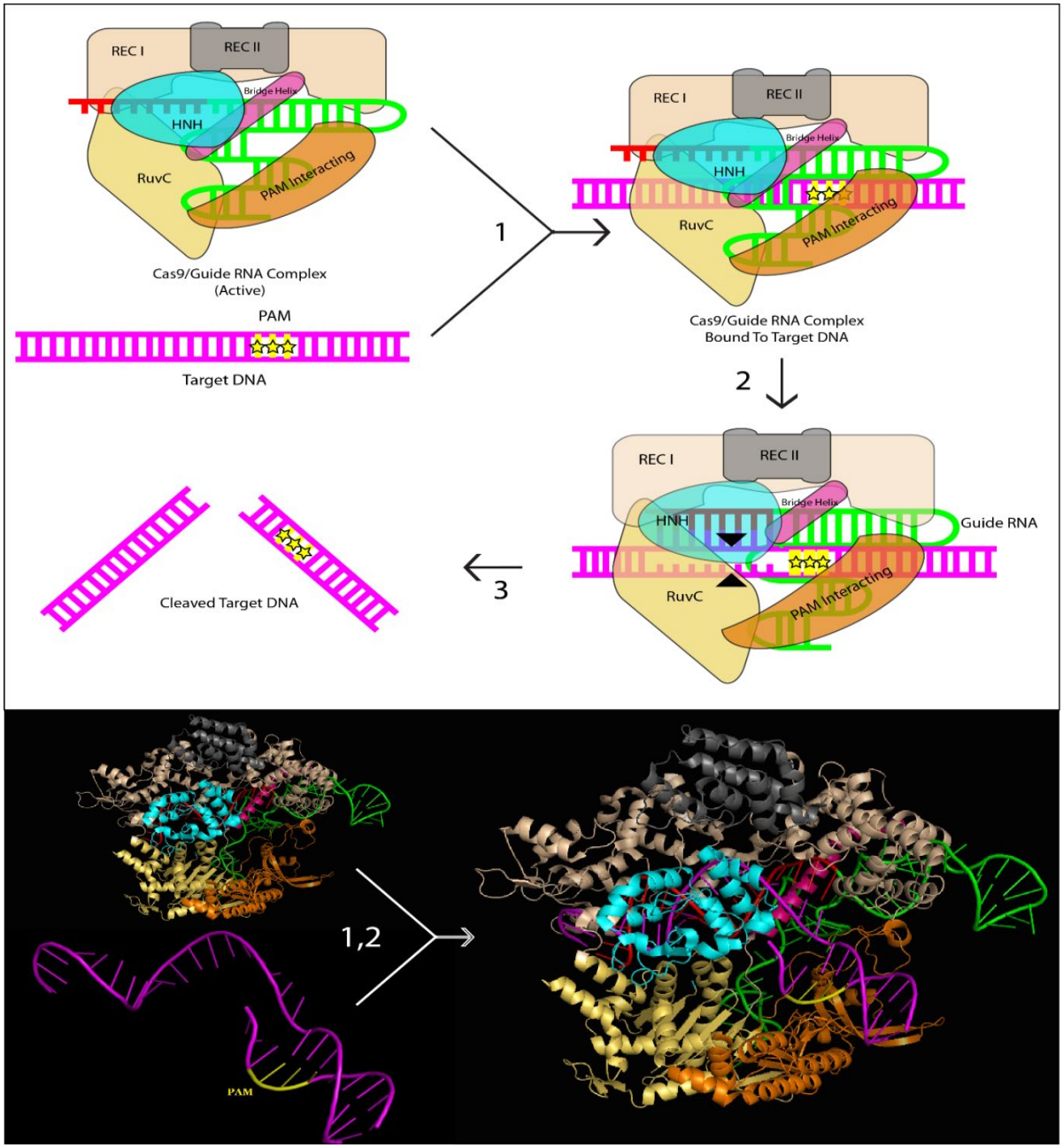

Figure 4. The sequence of actions of CRISPR-Cas9 [14].

2.3.3. Repair. The two processes for repairing double stranded breaks (DBSs) includes: non-homologous end joining (NHEJ) and homology-directed repair (HDR). In the NHEJ process, break ends are directly ligated without the use of a homologous template, whereas the HDR pathway utilises a donor DNA template to precisely repair the DNA break. NHEJ is substantially more prone to errors than HDR. Indeed, random mutations such as insertion or deletion at the cleavage site frequently result in frameshift or premature stop codon. This will disable the gene of interest, either turning it off or causing the synthesis of premature or non-functional polypeptide. NHEJ can thus be used to knock off genes. On the contrary, HDR is highly specific as it can repair defective genes and can be used to carry out gene insertion by adding a specific donor DNA template [15].

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3. Applications in cancer models

3.1. In vitro

CRISPR-Cas9 has been widely deployed in cancer research because it allows for genetic editing of practically all cell types. Scientists use CRISPR to generate cell lines with various genetic alterations, allowing them to investigate the genetic underpinnings of cancer and propose potential treatments. To slow cancer progression, this approach can target oncogenes or tumour suppressor genes (TSGs). Oncogenes are a class of genes related to cancer. In tumour cells, they are often mutated or overexpressed. The majority of oncogenes arise from proto-oncogenes, which are genes involved in cell growth and proliferation or apoptosis inhibition. Cell organoids are cell constructs with distinct tissue morphologies and functional characteristics differentiated from adult stem cells or embryonic stem cells, which can be utilised to create in vitro cancer models.

3.1.1. CRISPR/Cas9 activates tumour suppressor genes: P53, PTEN, RB1, and NF1. CRISPR-Cas9 has been used to create cancer models in order to determine the genetic origin of breast cancer by knocking out certain genes, in other words, the target genes are deleted or inactivated. Researchers demonstrated that P53, PTEN, RB1 and NF1 are essential tumour suppressor genes and their results suggested that inactivation of these genes will generate breast cancer [16]. Breast organoids were developed from normal breast epithelial subsets, which were then altered genetically using CRISPR/Cas9 to knockout the four tumour suppressor (P53, PTEN, RB1, and NF1) genes. The mutated organoids showed increased proliferation relative to controls and this resulted in the development of ER-α luminal breast cancer. This finding may have a profound impact as it presents a potential treatment strategy in the management of BC [17, 18].

3.1.2. FOXP. CRISPR-Cas9 technology can also be deployed to knock in genes in tumour cells, so as to up-regulate the expression of certain genes. FOXP3 is an X-linked key inhibitor of HERS-2/ErbB2 pathway. Compared with tumour suppressor genes on autosomal chromosome, tumour suppressor genes on X chromosome can be activated with a single action. Recently, researchers have developed a new method, in which X inactive-specific transcript (XIST) RNA, which is implicated in X chromosome silencing, is turned off while reactivating the FOXP3 gene. They accomplished this by transducing both XIST- and FOXP3-sgRNAs in breast cancer cell line HCC202 with heterozygous mutation of FOXP3. Their findings showed that cell growth was slowed [19].

3.2. In vivo models

Unlike in-vitro studies which are conducted in a controlled laboratory environment (e.g., in a petri dish or test tube), in-vivo models refer to studies which are conducted in living organisms. Numerous researchers have used in-vivo models to study the CRISPR-Cas9 system in cancer models and a selection of these articles are explored below.

3.2.1. Dual CRISPR interference. Cui et al. conducted a study in-vivo (in rodents) and in-vitro [19]. The same experiment as in 3.1.2 was repeated in vivo using female mice models with tumour cell injection. CRISPRi/a cells with FOXP3/XIST-sgRNAs and HCC202 cells without FOXP3/XIST-sgRNAs were injected into the tibia of female rats. The experiment used fluorescence imaging to demonstrate that this method can effectively delay cancer progression because the growth of CRISPRi/a tumours in bone tissue is much slower. This phenomenon was found in 60% of the mice in the CRISPR/a cell group (3 out of 5), but not in the control group (0 out of 5).

3.2.2. Chromosomal rearrangement. A chromosomal rearrangement is a type of chromosome abnormality which involves deletions, duplications, inversions and translocations. Typically, the rearrangement is resulted from a breakage in the DNA double strands, followed by a re-joining of the

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broken ends to generate a new combination of genes. This is a hallmark of oncogenesis and is present in several types of tumours.

In an in vivo rodent model, Blasco et al. developed a method using CRISPR-Cas9 to recapitulate chromosomal rearrangement present in human cancers in mice [20]. The chromosomal reversal of EML4-ALK caused the fusion of the amino terminal of EML4 and the enzyme domain of ALK [21]. This fusion activates ALK, a kinase, which in turn accelerates the proliferation of tumour cells. EML4-ALK gene mutation is expressed in 5-7% of NSCLC, which is a very common cancer [22]. They designed sgRNAs to target intron 13 and 19 in order to obtain the same chromosomal breakpoints found in human NSCLCs and generate EML4-ALK fusion in mice.

Their methodology was feasible as all mice developed EML4-ALK-rearranged tumours. This novel method using CRISPR-Cas9 to generate mouse models of gene fusion will be instrumental for the advancement of cancer research, allowing for the study of treatment response and resistance mechanisms. This study would not have been possible to conduct using knockin mice by traditional embryonic stem (ES) technology, as opposed to the CRISPR-Cas 9 method, the ES system is more time-consuming and economically inefficient [23]. Most notably, in ES models, researchers generate a specific germline mutation or modulate a specific allele in order to produce tissue-specific mutations, resulting in the entire organ carrying the same mutation. On the other hand, CRISPR-induced mutations exclusively target somatic cells, meaning the mutation does not affect all cells, which is what is seen in human cancers.

3.2.3. Human clinical trials. Rodent cancer models that used CRISPR-Cas9 have helped in the advancement of human in-vivo clinical trials related to cancer. Indeed, researchers at the UK hospital Great Ormond Street conducted a phase 1 trial in children with B cell leukaemia utilising CD19 targeting cells that met its safety criteria [24].

CRISPR Therapeutics is also conducting phase 1 trials with two types of allogeneic CRISPR-modified CAR-T cells [25]. The first is CD70 (which is frequently found on the surface of malignant cells in various lymphomas and solid tumours). The second treatment focuses on CD19, a protein found on malignant cells in leukaemia and lymphoma.

4. Advantages of CRISPR-Cas9 over other gene-editing methods

4.1. Zinc-finger nucleases (ZFNs)

ZFNs are artificial restriction enzymes which are formed by the fusion of a zinc finger DNA-binding domain to an endonuclease domain Fok1. The DNA target sequence is then approached by the highly specific DNA-binding domain, which in turn can induce double-stranded DNA breakages.

4.2. Transcription activator-like effector nucleases

The bacteria plant pathogen known as Xanthomona can be used to derive the transcription activator-like effector (TALE) protein. In a transcription activator-like effector nuclease (TALEN), the zinc finger protein can be replaced by a TALE protein from a bacterium. TALENs afford high specificity with a target site of 30-40 base pairs [26].

The CRISPR-Cas 9 complex has numerous advantages above and beyond the two aforementioned methods. Indeed, the CRISPR-Cas9 complex allows multiplexing, which means it can modulate multiple genes, which is helpful in the treatment of diseases which are polygenic (i.e., they involve many different alleles). Moreover, it is easier to design gRNA and the process is considerably cheaper [26].

5. Pitfalls of CRISPR in cancer models

5.1. Tumour heterogeneity

Arguably, one of the most significant disadvantages of employing the CRISPR system when inducing cancer is the efficiency of sgRNA when mutating multiple genes simultaneously. This results in the

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production of numerous clones with variable mutations, as some genes are not edited by the CRISPR system. However, this is a double-edged sword, as the imperfect repertoire of mutations generated by the CRISPR system means that unaffected cells can serve as control groups which can assist in data interpretation. An additional benefit of producing clones with variable genetic profiles is that clones with different mutations can be exposed to different selection pressures in keeping with Darwinian Evolution, which can better reflect human cancer pathophysiology [27].

5.2. Biased insertions and deletions

Genetic editing produced by the CRISPR system is random and investigators have little control over it. In general, insertions or deletions of single bases can result in frame shifts or a premature stop codon which can mean that the entire gene becomes disabled, hence amino acids cannot be sequenced and proteins cannot be synthesised. However, the insertion or deletion of three bases would mean that the amino acid sequence would not be affected [27].

5.3. Off target effects

The CRISPR system of Cas-9 has off target effects. Cas9 protein can generate cuts at non-target regions which can result in poor outcomes. Many efforts have been made to reduce the error rate. One way to reduce the non-target effect is to use Cas9 nickase which can cause a single strand cleavage. A double stranded break can be generated by Cas9 nickase with a sgRNA pair [28].

5.4. DNA damage toxicity

From time to time, the CRISPR-induced double strand breaks may trigger apoptosis. An example of this is a study in which p53 activation (introduced by the CRISPR system) in human pluripotent stem cells caused apoptosis as opposed to gene editing [28].

5.5. Immunotoxicity

The CRISPR/Cas9 system raises major issues regarding immunogenic toxicity. There is evidence that patients can have pre-existing anti-Cas9 antibodies against some of the most commonly utilised bacterial orthologs: SaCas9 and SpCas9 [28].

6. Conclusion

This review has addressed the mechanism of action of the CRISPR-Cas9 system in the context of gene editing. It has outlined the various vectors that can be involved in the delivery of the CRISPR-Cas9 system in-vivo and in-vitro and included evidence to demonstrate that the CRISPR-Cas9 system can be effectively employed in cancer models, with promising in vivo human data, but further research is needed to ensure that the limitations of the CRISPR-Cas9 system are stopped or reversed.

References

[1] Pan Shaowei, Zhang Huali. Discovery of CRISPR-Cas9 system[J]. Journal of Central South University(Medical Sciences),2021,46(12):1392-1402.

[2] MA Mengdan, SHANG Mengyu, LIU Yuchen. Application and prospect of CRISPR-Cas9 system in tumor biology[J/OL]. Synthetic Biology: 1-17[2023-06-25].

[3] TAN Lei, CHEN Mingyue, SHEN Bin. Journal of Nanjing Medical University (Natural Science Edition), 2021, 41(11): 1689-1694.

[4] DING Yidan, CHEN Chen. Research progress of CRISPR-Cas9 gene editing technology in tumor treatment[J]. Chinese Journal of Laser Biology, 2022, 31(06): 488-497.

[5] Matthew Behr, Jing Zhou, Bing Xu, Hongwei Zhang. In vivo delivery of CRISPR-Cas9 therapeutics: Progress and challenges[J]. Acta Pharmaceutica Sinica B, 2021,11(08): 2150-2171.

[6] SU Yuwen, Jian Bo, XU Qi. Establishment and comparison of in vitro cell models of blood-brain barrier[J]. Basic Medicine and Clinical Practice, 2022, 42(05): 714-720.

[7] ZHANG Yansong, CHEN Lijiao, ZHANG Ting, MA Yue, LI Shangang. Chinese Journal of Cell Biology, 2020, 42(10): 1858-1869.
[8] P. I. Ortinski et al., Selective induction of astrocytic gliosis generates deficits in neuronal inhibition[J], Nature Neuroscience, 2010,13(5):584–591.
[9] XU Yinyin, GU Zhuhui, QIAN Hai. Research progress of neoantigen cancer vaccine[J]. Pharmacy and Clinical Research, 2021, 29(04): 286-290.
[10] CAO Dayong, CHEN Bin, FU Jinfeng. Application of gene therapy in wound healing[J]. Chinese Journal of Injury and Repair, 2011, 6(06): 1012-1018.
[11] WANG Jing,SU Wen. Development history of glioblastoma and related molecular research progress[J].Journal of Practical Medical Technology,2022,29(12):1237-1240.
[12] Dai Ji, Zhang Tao. Progress in Biochemistry and Biophysics,2016,43(04):354-360.
[13] M. Yamashita and M. Emerman, Capsid is a dominant determinant of retrovirus infectivity in nondividing cells[J], Journal of Virology, 2004,78(11):5670–5678.
[14] C. &. Garrity, "CRISPR Mechanism," Tufts University, Available: https://sites.tufts.edu/crispr/crispr-mechanism/.
[15] LI Hegang, YANG Feng, ZHANG Baoxun, LI Peipei, WANG Jianhua, BAO Hanxun, MIAO Gang, JIANG Ke, LIU Nan. Research progress of CRISPR/Cas9 technology[J].Chinese Journal of Animal Science,2015,51(21):86-91.
[16] X. Xu, Y. Yuan, B. Feng, and W. Deng, CRISPR/Cas9-mediated gene-editing technology in Fruit Quality Improvement[J], Food Quality and Safety,2020,4(4):159–166.
[17] FANG Min,LI Li,LI Hengyu,SHENG Huang. Application and progress of CRISPR/Cas9 technology in the field of breast cancer research[J].World Clinical Drugs,2021,42(12):1156-1160.
[18] JIANG Shuchang, SHI Ming, PENG Hui. Application progress of CRISPR/Cas9 gene editing technology in tumor drug resistance research[J].International Journal of Pharmaceutical Research,2017,44(04):299-305.
[19] X. Cui et al., Dual CRISPR interference and activation for targeted reactivation of X-linked endogenous FOXP3 in human breast cancer cells[J], Molecular Cancer, 2022,21(1).
[20] R. B. Blasco et al., Simple and rapid in vivo generation of chromosomal rearrangements using CRISPR/Cas9 technology[J], Cell Reports, 2014,9(4): 1219–1227.
[21] Sui Zesen, Zhang Jianhua. Research progress on EML4-ALK fusion gene heteromorphism and its associated drug resistance[J].Medical Review,2020,26(12):2318-2322.
[22] LI Fei, HONG Chengyu, ZHENG Jian, GAO Hong, LI Xiaoling. Meta-analysis of the effectiveness of ALK inhibitors in the treatment of crizotinib-resistant non-small cell lung cancer[J].Modern Oncology,2021,29(16):2826-2832.
[23] YU Shumin, XU Xiaoming, HUA Jinlian, DOU Zhongying. Chinese Journal of Zoology, 2006(01): 128-133.
[24] G. Ottaviano et al., Phase 1 clinical trial of CRISPR-engineered car19 universal T cells for treatment of children with refractory B cell leukemia[J], Science Translational Medicine, 2022,14(668).
[25] "CRISPR therapeutics presents positive data on ALLOGENEIC CRISPR-based...," CRISPR, https://crisprtx.com/about-us/press-releases-and-presentations/crispr-therapeutics-presents-positive-data-on-allogeneic-crispr-based-car-t-cell-therapies-at-aacr-2018-2 (accessed Jun. 10, 2023).
[26] Yang Xinyu, Wang Dayong, Gao Xu. Application of gene editing technology and cell therapy in in vitro gene therapy [J]. Chinese Journal of Biochemistry and Molecular Biology, 2020, 36(11): 1265-1272.
[27] DONG Weipeng, WANG Junshi, ZHANG Shaohua, YAN Jiong. Research progress of CRISPR system and its application in mice[J]. Biotechnology Bulletin, 2018, 34(05): 57-63.
[28] Teng Xiaolong, Shi Shuobo. Optimization and development of CRISPR/Cas9 system in genome editing[J].Synthetic Biology,2023,4(01):67-85.