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A REVIEW ON GENOME EDITING BY CRISPR-CAS9 TECHNIQUE FOR CANCER TREATMENT

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Abstract – *In this review, we summarize CRISPR-Cas9 system-based gene modification for the therapeutic treatment of cancer. Cancer is a group of diseases involving anomalous cell growth with the potential to invade or blow out to other parts of the body, which is considered by not only multiple genetic but also epigenetic amendments that drive malignant cell propagation and consult chemo-resistance. The ability to correct or ablating such mutations holds enormous promise for battling cancer. Recently, because of its great efficiency and truthfulness, the CRISPR-Cas9 advanced genome editing technique has been extensively used in therapeutic investigations of cancers. Several studies used CRISPR-Cas9 technique for directly pointing cancer cell genomic DNA in cellular and animal cancer models, which have shown therapeutic potential in intensifying anticancer protocols. Moreover, CRISPR-Cas9 can also be engaged to fight oncogenic infections, discover anticancer drugs, and engineer immune cells and oncolytic viruses for immunotherapeutic treatment of cancer. We have been discussed the challenges and enhancements in translating therapeutic methods with CRISPR-Cas9 for clinical use. Therefore, in the study we suggested the potential directions of the CRISPR-Cas9 system for future cancer therapy.*

KEYWORDS: *CRISPR, Cas9, Cancer, Immunotherapy, HDR, Genome editing technique.*

INTRODUCTION

Cancer, a group of diseases involving unfamiliar cell growth having the potentiality to spread other parts of the body, is a leading life-threatening disease all over the world. Cancer cases will increase by 50% leading around 15 million deaths annually. In the United States alone, about 1.688,780 new cancer cases and 600,920 deaths were reported in 2017. By 2030, 26.4 million and 17 million deaths could occur[1]. Cancer is characterized by the accumulation of multiple genetic and epigenetic changes in the cancer cell genome, which drives cancer pathogenesis and development that disturb the cellular signaling and result in tumorigenic transformation and malignancy[2]. These changes include activated oncogenes (e.g. ErbB, RAS), inactivated tumor suppressors (e.g. p53, PTEN), mutations in epigenetic factors and their control loci (e.g. DNMT1), mutations in genes that confer chemoresistance and others. Cancer is currently one of the most serious diseases, which is challenging for human life and public health[1]. Although important progress has been made in cancer therapy, including surgery, radiation therapy, and chemotherapy, the high tendency of relapse and the primary or acquired chemoradiation resistance often result in poor diagnosis[3]. Therefore, the ability to correct or disable one or more sections of the cancer cell genome, for instance, recovering tumor suppressor genes function, may provide an interesting approach for cancer therapy, which can be done by genome editing[3]. CRISPR-Cas9 enabled researchers to edit the genome of eukaryotic cells more precisely and efficiently compared with others (ZFN & TALEN) [4,5].

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This system is adapted from bacteria and archaea as a defense mechanism for stopping foreign nucleic acids of viruses and plasmids. It can be selected to fight for oncogenic infections, exploring anticancer drugs, and engineering immune cells and oncolytic viruses for cancer immunotherapeutic applications. Due to resistance, conventional or chemotherapeutic treatment do not respond properly[6]. Therefore, the identification of new therapeutic targets is necessary to improve patient survival and clinical outcomes are crucial[7].

In this review, we discussed the potential directions of this system in cancer therapy and drug discovery. We also propose potential directions for this system for future cancer research.

HISTORY AND MECHANISMS OF CRISPR-CAS9 SYSTEMS

History of CRISPR-Cas9

In the mid-2000s, few microbiology and bioinformatics laboratories instigated considering CRISPRs, which had been designated in 1987 by Japanese researchers as a series of short direct repeats interspaced with tiny sequences in the genome of *Escherichia coli*[8]. Later CRISPRs were observed in various bacteria and archaea[9]. A key vision came in 2005 by the thought that many spacer sequences inside CRISPRs derived from plasmid and viral origins. From that time, this technique become so important by many researchers and it grasped in the cutting-edge stage (Figure 1).

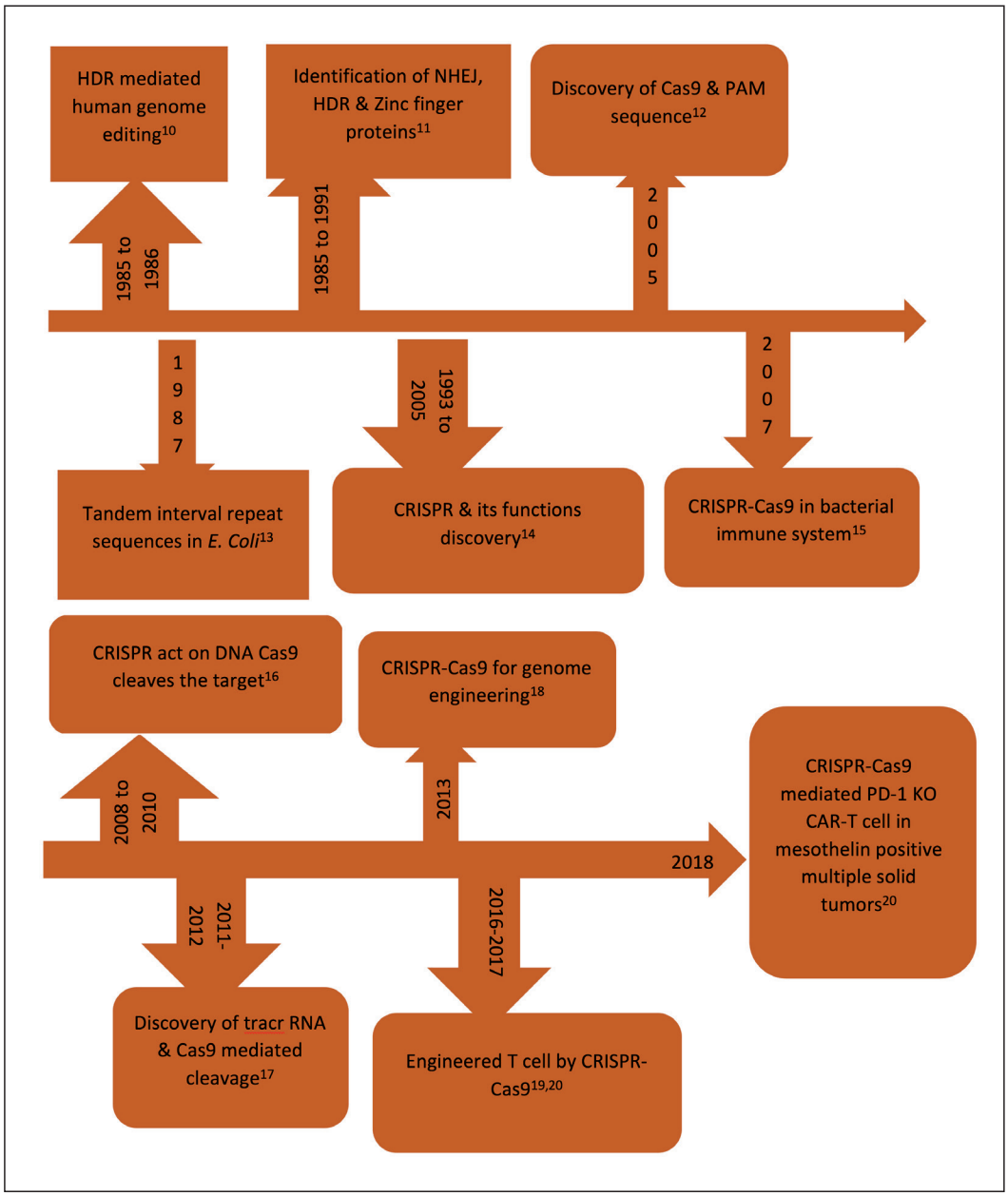

Fig. 1. Schematic representation of various stages in the discovery of CRISPR-Cas9 technology.

Mechanisms of CRISPR-Cas9

The CRISPR-Cas9 system was invented from the prokaryotic adaptive immune system[15]. Type II CRISPR-Cas system is a commonly used system consisting of three core components. The endonuclease Cas9, CRISPR RNA (crRNA), and Trans-activating crRNA (tracrRNA) [21]. The Cas9 is a protein responsible for detecting and cleaving target DNA. This protein contains six domains (i) REC I (ii) REC II (iii) Bridge Helix (iv) PAM Interacting (v) HNH and (vi) RuvC[22]. REC I is responsible for binding guide RNA. The arginine-rich bridge helix is critical for initiating cleavage activity upon binding of target DNA[22]. The PAM-Interacting dominion is consequently responsible for initiating binding to target DNA[22]. crRNA-tracrRNA duplex can be fused for forming a chimeric sgRNA for affluence of use in genome engineering[21]. Exactly, the sgRNA consists of a 20 nucleotide guide sequence complementary to the target site. When it recognizes the presence of a protospacer adjacent motif (PAM, typically 5'-NGG for Streptococcus pyogenes), it binds to the target sequence by Watson-Crick base-pairing and guides Cas9 to cleave the DNA strand forming a DSB at the target site. The RuvC and HNH nuclease dominions are responsible for cutting the target DNA following the third nucleotide base upstream of PAM. Subsequently, there are two major repair mechanisms for typically handling the breaks: Nonhomologous end-joining (NHEJ) and Homologous-directed repair (HDR) [23].

HDR uses a donor DNA template to precisely repair DSBs for gene modification with low efficiency, whereas the NHEJ repair mechanism frequently results in genomic insertions or deletions (indels) for gene disruption with high efficiency[24]. Normally, the NHEJ is error-prone and directly can join the break sequences, frequently introducing random insertions or deletions (indels) at the DSB site, thereby disrupting or eliminating target genes function by inducing frame shifts[24]. By combining the expression of sgRNA and Cas9, high-efficiency cleavage of any target sequence can easily be achieved[25].

CRISPR-CAS9 is outstanding than other techniques

The CRISPR/Cas9 system has several advantages over two other established genome editing techniques in terms of its simplicity, flexibility, and affordability. The most important difference among them is the CRISPR system relies on RNA–DNA recognition, rather than protein– DNA-binding mechanism[21,26]. For this reason, it is more 'feasible' and easier to construct a customized CRISPR/Cas9 complex by only changing the sequence of gRNA instead of engineering a new protein. The target sequence needs to be immediately upstream of a PAM sequence because the latter is essential for target recognition by Cas9. In the human genome, this short sequence occurs approximately once every eight base pairs, making possible to design several gRNAs for one specific target gene[27]. ZFN is the oldest and most established technique with low efficiency and also expensive than any other technique of genome editing. The main problem is the necessity of recoding large segments for every new target site. Both TALEN and CRISPR provide high target site specificity enabling researchers for making precise genetic alterations. CRISPR achieves this specificity through the sgRNA, an artificial fusion of two naturally occurring short RNAs[21]. CRISPR is popular because of its capability of modifying chromosomal targets at high frequencies. The rates of indel formation by CRISPR-Cas9 (more than 70%) have been reported. TALEN is also able to modify chromosomes with a high rate of efficiency but indel formation is lower (33%) than CRISPR-Cas9[27,28]. TALEN is sensitive for methylation, but CRISPR-Cas9 is not sensitive to methylation.

Potential application for therapeutic treatment of cancer

The development of the CRISPR-Cas9-mediated genome editing tool has perverse the field of gene therapy, which not only holds widespread application potential for therapeutic manipulations of cancer genomes, but also can be used for fighting oncogenic infections, modulate gene expression, and explore anti-cancer drugs. To directly target cancer cell genomes, the CRISPR-Cas9 system can also be applied for accurate engineering of immune cells and oncolytic viruses for cancer immunotherapeutic applications.

Future direction

With the marvelous improvements of the CRISPR-Cas9 technique, we can imagine several other potential instructions for applying the system in cancer therapy. Examining the feasibility of combining this molecular therapeutic strategy with radiation, traditional surgery or chemotherapy may be useful for increasing anticancer efficacy. Radiotherapy is one of the most important modalities which is used for treating cancer patients. Though, it has been exposed that tumors that contain some gene mutations, such as the p53 and p21 mutations, have poor sensitivity of radiation, often resulting in failure of radiotherapy[48].

TABLE 1. Potentiality of CRISPR Cas9 for cancer treatment.

Potential Application	*References*
CRISPR-Cas9 can be used for correcting gene aberrations that drive cancer pathogenesis	3
This system can function in a cancer cell-specific manner	29
CRISPR-Cas9 can Delete MCL-1 in human BL cells which causes apoptosis of BL cells.	30
It can target CDK11 knockout that may be a novel method treatment of osteosarcoma	31
knockout of SHCBP1 plays an important role in stimulating breast cancer cell apoptosis	10
It can reestablish the tumor suppressor proteins p53 and p21.	18
Genetic modification of T-cells can be used as a treatment of complex types of cancer.	32
It can be used for gene repairing or to inducing overexpression of tumor suppressor genes	33
Targeting gene transformation of T-cells is possible by CRISPR/Cas9.	34
It can effectively target and reduce the expression levels of specific miRNAs.	35
CRISPR-Cas9 can be used as a promising intervention strategy for cervical cancer.	36
It can target HCV RNA to prevent viral protein construction and duplication	37
It can be used to improve the selectivity of OVs to cancer cells in oncolytic virotherapy	3
TK gene was professionally and readily changed with the RFP gene by this system	38
Removal of PD-1 may be a very useful method to increase the efficiency of T-cell-based immunotherapy for treating cancer	39
Use of CRISPR/Cas9 to identify and delete the genes controlling tumor drug resistance.	40
Can effectively inhibit tumor cell growth by targeted deletion of oncogenes	33
F-LP/gDNMT1 can be promising cancer therapeutics using CRISPR-Cas9 technology.	41
CRISPR/Cas9 can specifically target mutant cancer cells, results in the selective elimination of mutant cells and reduced cell proliferation.	42
Elimination of EGFR mutant alleles (L858R) in an NSCLC cell line (H1975) represents cancer cell death and significantly reduced tumor size	43
CRISPR-mediated knockout of CXCR2 (IL-8 receptor) in breast cancer cells.	44
CRISPR-Cas9 system holds immense therapeutic potential for improving T cell-based immunotherapy	45
It can enhance the CAR-T cell effector function by disrupting PD-1 protein	46
CRISPR-Cas9 can induce mutations in the E6 gene responsible for cervical cancer cells	47

*Data are expressed as the mean ± SD (n=3).

The use of the CRISPR-Cas9 system to exactly correct these mutations in cancer cells or to interject the cellular radiation injury repair pathway may be a different method for increasing radiosensitivity. A combination of CRISPR-Cas9-mediated gene therapy and radiotherapy with synergistic anticancer effects may become a gifted avenue for cancer therapy. Another attractive therapeutic strategy that may be worth exploring is the binding of the CRISPR-Cas9 system for adjusting the cancer microenvironment for therapeutic purposes. Cancer stroma cells like cancer-associated fibroblasts, immune cells, and angiogenic vascular cells cooperate with cancer cells, and they contribute to cancer growth and development[49]. These non-tumor cells are less heterogeneous in comparison with malignant cells for this they become a new target for anti-cancer therapy and also shown clinical benefits[50]. For instance, targeting receptors like VEGF and VEGFR2, in angiogenic vascular cells with small molecular inhibitors or immune checkpoint protein, which is known as CTLA-4 in T cells with monoclonal antibody, have been used in multiple cancers. It can also be hazarded that CRISPR-Cas9 systems can be used for targeting cancer stroma cells for generating LOF mutation in VEGFR or CTLA-4. Another strategy may be used to improve tissue-specific cancer therapy in combination with CRISPR-Cas9. Cas9 only was expressed in bladder cancer cells and specifically, it is inhibited the growth of bladder cancer cell[28]. This promising strategy can be prolonged to other cancers. Furthermore, delivering the CRISPR-Cas9 system which is coated with specific ligands is responsible for targeting tumor-specific cellular receptor maybe another approach for increasing the delivery efficiency and specificity. The system would have significant insinuation in personalized therapy. It offers an experimental platform for determining the function of unclear pathogenic mutations or resistance mechanisms for individual patients rapidly, facilitating the expansion of strategies for achieving therapy for a given patient which

is effective. The possible clinical applications of the CRISPR-Cas9 editing strategy for correcting or destroying the mutated EGFR in lung cancer have been planned[51]. It is still a long distance to realize the use of CRISPR-Cas9-based genome editing as a therapeutic strategy adequately to target the cancer genes in human patients. It should be remembered that the use of (epi) genome manipulation therapies in cancers is imperfect by the mutation heterogeneity of the cancer cells. Further studies will be needed for increasing editing efficiency and reducing the off-target effects of this method. Similarly, to ensure that every cancer cell accepts Cas9-sgRNAs, an appropriate ratio of the amount of Cas9-sgRNA complex per cell genome must be recognized.

DISCUSSION

In this review we attempt to figure out the use of CRISPR-Cas9 technique for cancer treatment, and we also investigated the comparison among CRISPR-Cas9 and other established genome editing techniques. CRISPR-Cas9 is the most advanced and better technique than others. It is popular because of its capability to modify chromosomal targets at high frequencies. The rate of indel formation by CRISPR-Cas9 is more than 70%, which is greater than ZFN or TALENs[28]. The CRISPR-Cas9 not only holds pervasive application potential for therapeutic manipulations of cancer genomes but also can be used for fighting oncogenic infections, modulate gene expression, and explore anti-cancer drugs. To directly target cancer cell genomes, the CRISPR-Cas9 system can also be applied for accurate engineering of immune cells and oncolytic viruses for cancer immunotherapeutic applications. One limitation of CRISPR-Cas9 is the size of the Cas9 protein (4.2 kb) that makes it challenging to deliver. Another major problem regarding CRISPR-Cas9 is the off-target effects. These off-target effects are guided RNA specific. It has been recommended that alternative CRISPR-Cas9 systems may offer improved targeting specificity. In recent years, a significant progress has been made in delivery methods for this technique and other nucleic acid-based therapeutics. Completely new or enhanced delivery methods will be needed for making CRISPR-Cas9 technology suitable for human applications. It is possible to increase targeting efficiency by discovering the new CRISPR-Cas9 system from the defense system of new species containing PAM sequence different from established. If a longer PAM sequence of 5'-NNNNGATT-3' can be discovered, it will be possible to increase the targeting efficiency by more than 60%. Therefore, additional work will be needed for this system.

CONCLUSIONS

In summary, therapeutic strategies based on CRISPR-Cas9 holding enormous therapeutic potential for increasing our anticancer approaches, although there are still some open challenges. Compared to detailed (epi) genome modifications, gene editing for epigenetic inflection and transcriptional regulation or engineering genetic modified OVs and immune cells may be fewer challenging for cancer therapeutic applications. Further optimization and development of CRISPR-Cas9-based gene editing, especially for eliminating the off-target effects and increasing the efficiency of genome editing and delivery of cancer cells, are needed to satisfy the necessities of therapeutic application. Continual advances in CRISPR-Cas9 technology will allow its therapeutic use for treating cancer in clinical patients.

Conflict of Interest:
The authors declare that they have no conflict of interests.

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