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A Review on CRISPR/Cas9 as a Novel Technique for Cancer Therapy

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Summary

Cancer is a disorder that, basically, occurs as a result of genetic and epigenetic abnormalities. It's one of the leading causes of death in the globe, and it's still a major social and economic problem. According to statistics, over 10 million people die with malignancies, and cancer rates are expected to increase by 50% in the next ten years, culminating in approximately 15 million deaths. Single or multiple gene mutations, chromosomal abnormalities may cause cancer. Although numerous treatment options are used to treat cancer, they are still insufficient against malignant diseases. Therefore, a variety of novel strategies for early cancer therapy are examined. One of the most recent and potentially effective technologies that has been used in last years for genetic modification and cancer therapy is Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-associated protein-9 (Cas9), a unique RNA domain-containing endonuclease-based genome engineering technology. In simple words, CRISPR/Cas9 has been derived from a bacterial defensive mechanism against viral infection. Recently, this approach has proved its usefulness in cancer therapy and gene editing. In general, this report presents a review of this key technology and its components. Specifically, in this work, we address the probable prospective uses and recent breakthroughs of CRISPR/Cas9 technology in cancer treatment, as well as the problems that can be encountered during clinical investigations. In this regard, we intend to contribute to optimizing work on CRISPR/Cas9 as well as to focus on the probable future paths of this technology.

Keywords

CRISP, Cas9, modern technology, cancer, therapy.

Introduction

Cancer is a broad term for a series of diseases characterized by irregular cell development with the ability to infiltrate and disseminate to other body parts [1]. It's one of the most common causes of death worldwide and a significant public health issue. In 2020, 19.3 million new cases of cancer and over ten million deaths from cancer were registered, globally [2].

Cancer is featuring by the aggregation of many genetic and non-genetic alterations in the cancer cell genome, which lead to carcinogenesis and malignant growth [3]. These alterations may include inactivated tumor suppression, oncogene activation, epigenetic factor mutations, and chemoresistance mutations [4].

Despite the significant advancements in cancer treatment, such as irradiation, chemotherapy, and surgery, the high likelihood of rejection and primary or acquired chemo-radiation tolerance usually leads to inadequate treatment [5]. As a result, the ability to repair or destroy certain DNA regions of a cancer cells which can be achieved by genome editing, can provide an important method for cancer therapy [5].

Genome editing is a kind of genetic modification in which artificially modified nucleases or molecular scissors are used to insert, substitute, or delete DNA from a genome [6]. However, the gene editing technologies are divided into three methodological generations: zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regulatory interspaced short palindromic repeat (CRISPR) system [7].

CRISPR is an adaptive immune system in bacteria that comprises a bank of foreign genetic information and a process for identifying and killing the invading foreign agents like plasmids and viruses [8]. The CRISPR systems are found in 70% of bacteria and 90% of *Archaea*, and some contain several CRISPR areas on their chromosomes. However, following discovery of CRISPR system as a natural defensive mechanism in bacteria, the researchers tried to modify it to make it a useful tool for gene editing [9]. The CRISPR system comprises a single guide RNA (sgRNA) that targets the specific gene and the Cas9 protein, which is now the most widely used gene editing tool [9]. Moreover, the CRISPR technology has been used in oncology testing and cancer therapy trials since it allows for accurate and effective genome engineering [10, 11].

CRISPR Background

In 1987, CRISPR was first discovered in *Escherichia coli* when researchers were looking for the gene that controls alkaline phosphatase isozyme conversion [12]. Also, CRISPRs were discovered in *Archaea*, especially *Haloferax mediterranei*, in 1993, and have subsequently been found in multiple bacterial and archaeal genomes [13].

In the mid-2000s, the discovery of similarities between the spacer regions of CRISPRs and the succession of archaea, plasmids, and bacteriophages provided an insight that CRISPRs could play an essential role, e.g., in immune system [14]. Later, in 2002, Cas (CRISPR-Linked) genes were assigned to genes that were predicted to encode DNA repair proteins for hyperthermophilic *Archaea* and were found to be strongly associated with CRISPR [15]. Meanwhile, CRISPR is a term has been universally launched. Similarly, in the eukaryotic RNA interference (RNAi) system, comparative genomic studies have suggested that CRISPR and its proteins function together, forming a supposed immunity mechanism to protect prokaryotic cells from invading pathogens and plasmids [16]. Spacer repeats are transcribed into CRISPR RNAs (crRNAs) that lead the Cas enzyme to the invader's target DNA [17]. In 2012, Jennifer Doudna and Emmanuelle Charpentier proved that the CRISPR-Cas9 can be programmed with RNA in order to edit genomic DNA [18]. However, the use of CRISPR/Cas9 in the modification of human genomes was then declared, thus paving the way for CRISPR use in medicine [19]. Moreover, in 2016, CRISPR/Cas9 modified immune cells were utilized in order to treat people with lung cancer in the first human clinical study using CRISPR [20]. In 2020, for their development of CRISPR/Cas9 technology, the Nobel Prize in Chemistry was given to Emmanuelle Charpentier and Jennifer Doudna. Figure 1 shows, in brief, the time course of CRISPR technology evolution.

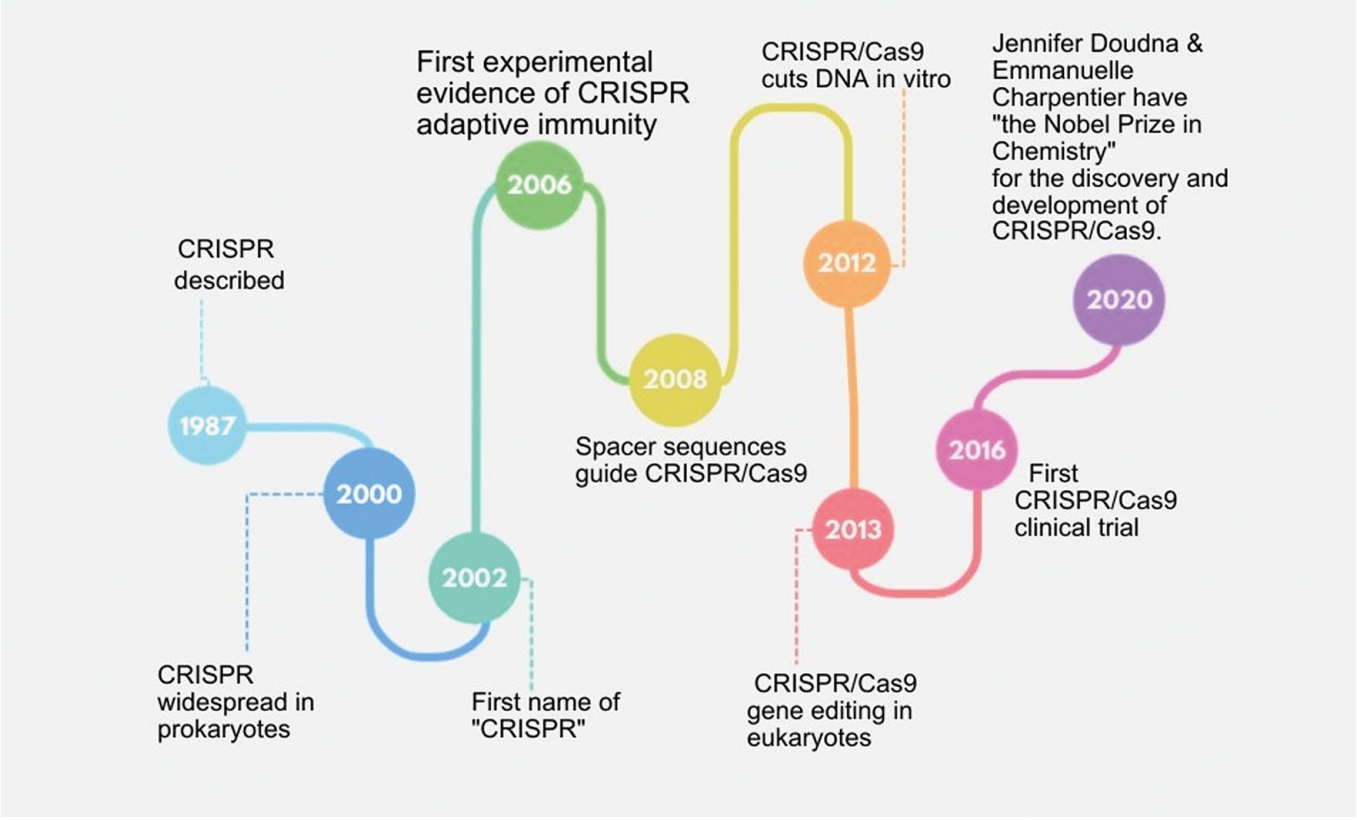

Figure 1. Timeline of the CRISPR technology evolution

CRISPR/Cas system classification

There are two classes of CRISPR systems that are divided into six different types and several subtypes. Class 1 includes I, III, and IV types. While class 2 includes II, V, and VI types, being classified according to structural and functional properties [Table 1]. Also, the CRISPR system contains many associated proteins with distinct type of CRISPR [Table 2]. The CRISPR/Cas class 1 system employs a mixture of many Cas proteins, while the class 2 system only employs one Cas protein with several domains. Therefore, the class 2 CRISPR/Cas system is preferable for gene engineering due to its easiness and simplicity. The type II CRISPR/Cas9 system is the most commonly used and studied among the different types of CRISPR class 2 systems. [8, 21].

CRISPR/Cas9 as an editorial tool

Cas9 is a CRISPR protein type II, class 2, targets DNA molecular. It is a crRNA-guided endonuclease with HNH and RuvC nuclease regions that cleaves the genomic dsDNA [18]. The HNH nuclease region splits the strand of DNA complementary to the gRNA array, whereas the RuvC nuclease region splits the strand of DNA [18]. The most frequently used type of CRISPR/Cas9, *Streptococcus pyogenes* Cas9 (SpCas9), targets DNA by recognizing the protospacer adjacent motif (PAM) [22]. The Cas9 protein size is variable for different bacterial species, with 1053 amino acid residues (a.a) in *Streptococcus aureus* and 1368 a.a in *Streptococcus pyogenes* [23]. The CRISPR/Cas9 system is composed of crRNA, tracrRNA, and Cas9. Artificially, tracrRNA and crRNA can be turned into sgRNA, which guides Cas9 to the target region [24].

Table 1. Classes of CRISPR system [8, 21]

CRISPR class	Type	Subtypes	Associated Protein	Target	General Characteristics
1	I	I- A, B, C, D, E, F and U	Cas1, 2, 3, 4, 5, 6, 7, 8, 10	DNA	Requires a PAM sequence.
	III	III-A, B, C, D	Cas1, 2, 5, 6, 7, 10, Csm, Cmr	RNA and DNA	- Make single-stranded nicks for both RNA and DNA targets. - A PAM repeat is not needed.
	IV	None characterized	Cas1, 5, 7	DNA	Many preserved Cas genes are missing, as well as a CRISPR array.
2	II	II- A, B, C	Cas1, 2, 4, 9, RNase 111	DNA	- Both tracrRNA and crRNA are needed. - Makes double-stranded nicks in the target DNA. - A PAM repeat is not needed.
	V	V- A, B, C	Cas2, 4, 12	DNA	- Makes double-stranded fractures in the target DNA. - A PAM repeat is needed. - Both crRNA and tracrRNA are needed by the Type V-B effector (C2c1).
	VI	VI- A, B, C	Cas1, 2, 13	RNA	- Makes single-stranded nicks in target RNAs. - A PFS is needed.

Abbreviation: PAM: Protospacer adjacent motif, crRNA: CRISPR RNAs, tracrRNA: Trans-activating crisper RNA.

Table 2. Proteins in CRISPR system [8, 21]

CRISPR Protein	Function
Cas1	DNA nuclease
Cas2	RNA nuclease
Cas3	DNA nuclease and helicase
Cas4	DNA nuclease
Cas5	Ribonuclease responsible for converting pre-crRNA to mature crRNA.
Cas6	Ribonuclease responsible for converting pre-crRNA to mature crRNA.
Cas7	It has an RNA identification motif and joins crRNA, which is usually found in multiple copies.
Cas8	Big subunit of effector component in type I
Cas9	DNA nuclease
Cas10	Big subunit of effector component in type III
Cas12 (Cpf1)	DNA nuclease, crRNA processing
Cas13 (C2c2)	RNA nuclease, crRNA processing
Csm, Cmr	RNA nuclease and single-stranded DNA
RNase III	Processes tracrRNA and promotes with crRNA maturation

Mechanism of CRISPR/Cas9 system action

CRISPR is a natural defense mechanism that helps bacteria and *Archaea* to resist viral or exogenous plasmid invasion [25]. When a virus infects bacteria, remnants of the viral DNA are embedded into the bacterial CRISPR gene, thus serving as a memory. I.e., when the same virus infects the bacterium again, it can recognize the virus by using this marker. Moreover, bacteria use the Cas9 endonuclease to trigger a double-strand break (DSB) in the viral DNA, which can result in viral inactivation [26]. At the molecular level, the mechanism of CRISPR-Cas9 action can be presented into three major phases: Adaptation, Biogenesis, and Interference, as illustrated in Table 3.

At the technical level, the CRISPR type II system is made up of the Cas9 protein and single guide RNA (sgRNA). Cas9 acts as a nuclease that triggers DSBs in the DNA molecule, while sgRNA can identify the target site, particularly through homologous recombination of the 20-bp DNA sequence [30, 31]. Thus, when the CRISPR/Cas9 system is introduced into a cell, the gRNAs direct the Cas9 nuclease to a particular DNA site with a protospacer adjacent motif (PAM) that corresponds to the gRNA. Then, the Cas9 nuclease breaks the DNA double strands and produces a DSB [32]. As shown in Figure 2, an endogenous repair mechanism, e.g., non-homologous end joining (NHEJ) and homology directed repair (HDR) can mostly repair the DSBs caused by Cas9 nuclease [33]. NHEJ is effective but not precise and could cause genetic mutations such as deletions or insertions [34]. Meanwhile the HDR path is ineffective and proceeds through mitosis only. However, HDR allows for precise DNA repair based on homologous sequences [35]. Notably, the CRISPR/Cas9 system is used to edit genes in a variety of cells, and successful transfer of the CRISPR-Cas9 system into cells is still a major challenge.

Table 3. The three phases of CRISPR/Cas9 mechanism [27, 28, 29]

Adaptation	Biogenesis	Interference
When a virus infects a microbe, like a bacteria or archaea, the immune system captures and incorporates a piece of the invading virus's DNA into its own genome in the shape of a "spacer" at the CRISPR locus. Subsequently, it acts as a virus's memory, allowing microbes to program their defenses against related phages in the future. As a result, these spacers are used to destroy viral particles as part of their defensive response [27].	In this phase, the CRISPR sequence is transcribed, resulting in a long precursor CRISPR-RNA (pre-crRNA). This pre-crRNA is a long transcript with a spacer and a repeat chain, which is processed further with another type of RNA called tracrRNA (trans-activating crRNA) to form the crRNA. This crRNA contains the invader's genome code, which is later used to signal the Cas protein to cut off the phage or plasmid DNA [26, 28]	Interference is the final stage of the CRISPR mechanism. It includes cutting phage genetic material with the aid of the crRNA/tracrRNA duplex and the Cas9 molecule [29]

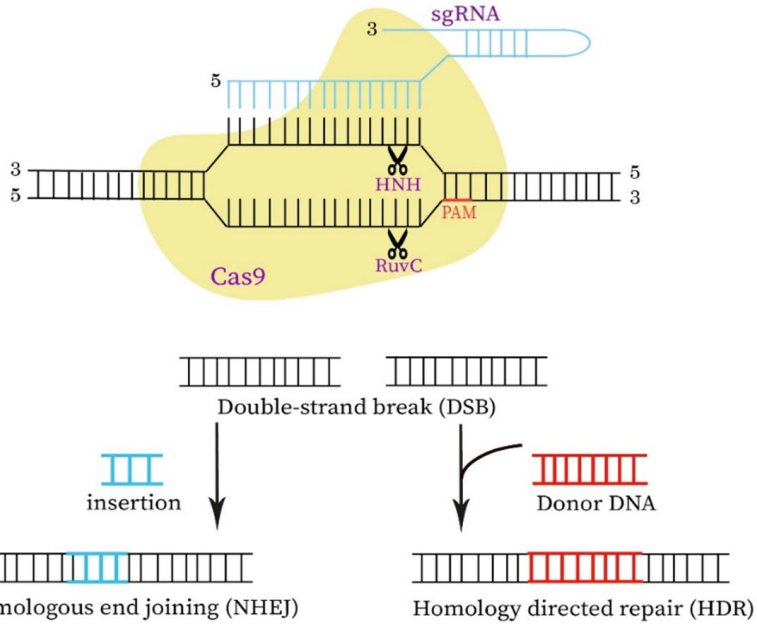

Figure 2. Endogenous repair mechanisms; non-homologous end joining (NHEJ) and homology directed repair (HDR)

Delivery systems of CRISPR/Cas9

There are several CRISPR/Cas9 genome editing strategies: sgRNA and Cas9-mRNA, sgRNA and Cas9 protein, and a plasmid-based CRISPR-Cas9 system [36]. The benefits and disadvantages of these strategies are illustrated in Table 4. However, the efficient distribution of the CRISPR/Cas9 system to cancer cells is essential for the CRISPR/Cas9 system to be successful in treating cancer. Therefore, the CRISPR/Cas9 system for cancer gene treatment has been studied using three delivery approaches: physical approaches, non-viral vectors, and viral vectors.

Physical methods of gene transduction

Physical methods do not depend on the utilization of vectors, but rather on making pores in the cell membrane [38]. However, the physical method provides a delivery process that is unaffected by the type of cell or package size [39]. Electroporation is a common physical method used to deliver CRISPR/Cas9 with great efficiency. It employs electrical current pulses to promote transient holes in plasma membranes, allowing the cargo to be delivered into cells [40]. On the other hand, *in vitro* electroporation has successfully delivered a CRISPR/Cas9 plasmid into cancer cells [41, 42]. Despite the benefits of electroporation, cell damage induced by electroporation may be a major concern for *in vitro* experiments [40]. Moreover, the delivery of CRISPR/Cas9 may be performed by other common physical methods, e.g., microinjection, membrane deformation, and hydrodynamic injection [43, 44]. Herein, Table 5 shows some studies that used physical methods to deliver CRISPR/Cas9.

Viral vectors

Viral vectors are broadly utilized as gene delivery tools because of their great effectiveness and potentially, long-term effects due to their integration with the host DNA [50]. However, there are various viral types for CRISPR/Cas9 delivery, i.e., adenovirus (AdV), retroviruses (RV), adeno-associated

Table 4. Features of various CRISPR/Cas9 genome editing strategies

Strategies	Advantages	Disadvantages	Article
sgRNA and Cas9-mRNA	Low off-target effects, rapid onset, and transitory expression	Poor stability	[36, 37]
sgRNA and Cas9 protein	Rapid onset, short duration, low off-target effects, and high efficiency	Endotoxin contamination and high prices	
plasmid-based CRISPR-Cas9 system	Good stability and low price	Inefficiency, integration issue, a late onset	

Table 5. CRISPR/Cas9 delivery by means of physical methods

Study type	Type	Carried substance	Cell Line	Action	Article
In vitro	Electroporation	CRISPR/Cas9 plasmid	Human osteosarcoma U2OS cells	repressed CDK11 expression	[41]
			Human myeloid leukemia KBM5 cells	repaired ASXL1 gene expression	[42]
	Membrane deformation	• Cas9 and sgRNA EGFP • Cas9 and sgRNA-AAVS1 • Cas9 and sgRNA-NUAK2	• MDA-MB231 cells • MCF7 cells • Human cervical HeLa cells	• EGFP knockout • Split at the AAVS1 locus with indels • NUAK2 indels	[43]
	Ultrasound-propelled nanomotors	Cas9/sgRNA complex	Murine melanoma B16F10 cells	GFP knockout	[45]
	lance array nano injection (LAN)	CRISPR/Cas9 plasmid	GFP+/FRT HeLa cell	Disruption of EGFP gene	[46]
Ex vivo	Electroporation	CRISPR/Cas9 plasmid	Human primary T cell	PD-1 gene knockout	[47]
		Cas9/sgRNA complex	Human primary B cell	secretion of therapeutic proteins	[48]
		Cas9/sgRNA complex	Human primary NK cell	TGFBR2 and HPRT1 genes knockout	[49]
In vivo	Hydrodynamic injection	CRISPR/Cas9 plasmid	M-TgHBV mouse model	Remove two open reading frames (ORFs) of HBV reproduction template	[44]

virus (AAV), lentivirus (LV), Epstein-Barr virus, Sendai virus, and baculovirus. The loading capacity of viruses is variable (4.7-38 kb), thus defining the package of genes encoding the CRISPR/Cas systems enzyme [39]. However, AAVs have mostly been employed for CRISPR genome editing *in vivo* due to their unique features, e.g., being less immunogenic, having low toxicity, and having many AAV serotypes [51]. On the other hand, lentivirus (LV) is often used to deliver CRISPR/Cas9 *in vitro* because of its capacity to permeate the nuclear membrane without causing cell division [39]. Table 6 depicts some trials that used viral vectors to deliver CRISPR/Cas9.

Non-viral vectors

CRISPR/Cas9 may also be introduced to the cells using non-viral vectors. These approaches provide lower immune response, are not restricted by packaging limits, are simpler to synthesize, and can deliver many sgRNAs at once [50]. Furthermore, compared to viral vectors, non-viral vectors have fewer off-target effects [58]. Non-viral vectors, on the other hand, have limited *in vivo* applications due to their low transduction efficiency, despite their safety and ease of use [51]. Table 7 shows some trials that used non-viral delivery systems to introduce CRISPR/Cas9.

CRISPR/Cas9 Applications

CRISPR/Cas9 technology has paved the way for novel opportunities in human gene editing. Recently, it has been used in a variety of areas, including the treatment of genetic diseases, detection of disease- related gene and diagnosis, tumor therapy, genetic engineering of plants and animals, and the suppression and management of harmful bacteria [65].

Table 6. Different viral vectors used for the *in vitro* CRISPR/Cas9 delivery

Study type	Virus	Target gene	Cell line	Action	Article
In vitro	Adeno-associated virus (AAV)	HPV-E6 protein	HCC cell lines (HeLa, HCS-2, SKG)	Increase p53 expression, apoptosis, and inhibited development	[52]
	Lentivirus	E6- or E7-specific sgRNA	HCC Hela cell	HPV E6 or E7 inhibition in HeLa cell	[53]
Ex vivo	AAV	VEGFR2	HRE-Cells	Inhibition of VEGFR2	[54]
	Lentivirus	MUC18	Human primary nasal airway epithelial cells	MUC18 knockout	[55]
In vivo	Lentivirus	HIF-1α	SMMC-7721 xenograft HCC model	HIF-1α knockout	[56]
	Adenovirus	EGFR gene	H1975 cells	EGFR gene disruption	[57]

Table 7. CRISPR/Cas9 delivery *via* non-viral vectors

Study type	Type	Target	Cell line	Action	Article
In vitro	X-tremeGENE HP DNA Transfection Reagent	E7 gene	Human cervical cancer Siha cells	Increase apoptosis and inhibit the viability of cells	[59]
	flexible dendrimer	MASPIN gene	Human breast cancer MCF-7 cells	Activation of MASPIN suppressor	[60]
Ex vivo	LPEI	Mesenchymal stem cells (MSC)	Mesenchymal stem cells	Highly efficient cytokine overexpression	[61]
In vivo	PLNP	Plk1 gene	Subcutaneous A375-derived melanoma	Reduce Plk1 protein expression; decrease tumor progress.	[62]
	SKOV3 cell-derived exosomes	PARP-1 gene	SKOV3 cell	PARP-1 suppression; increase the chemosensitivity to cisplatin	[63]
	Folate-modified liposomes	DNMT1 gene	SKOV3 cell	Reduce DNMT1 expression; decrease tumor growth	[64]

CRISPR/Cas9 Application in cancer therapy

Despite been some advances in recent years, the rate of deaths due to cancer continues to rise, demonstrating the essential need for new and more effective treatment approaches. CRISPR/Cas9 technology seems to be a potential tool for cancer treatment. Due to its multiple applications in targeting cancer cells, such as cancer immunotherapy, oncolytic virotherapy, stromal-targeting therapies, etc. The CRISPR/Cas9 technology could be a promising tool of cancer treatment [66]. By using a variety of CRISPR/Cas9 strategies such as base editing and gene knockout/in, CRISPR/Cas9 can be utilized to replace, remove, or correct undesirable genes that cause genetic diseases [32]. Moreover, CRISPR/Cas9 is used in the treatment of different types of cancer such as lung, breast, liver, and others malignancies.

1. Lung cancer

Lung cancer is the major cause of fatality-related cancer in both men and women [2]. Various genes like EGFR, CD38, FAK, RSF1, and others are thought to be proto-oncogenes linked to lung cancer. Likewise, GOT1, MFN2, miR-1304, and others are recognized as suppressor genes in this malignancy [67]. The overexpression of oncogenes and suppressor gene mutations may promote the tumor development. In this respect, the CRISPR/Cas9 technology has the potential to effectively eradicate lung cancer [68]. By targeting the oncogenes CD38 and KRAS, CRISPR/Cas9 knockout/down decreased cell proliferation and tumor growth *in vivo* [69, 70]. Moreover, the knockout of the MFN2 suppressor gene enhances cell activity and colony formation by activating the mTORC2/Akt pathway [71]. Another study found that knockout of the suppressor gene Plakophilin 1 (PKP1) in the A549 cell line increased cell dissemination while decreasing their reproduction [72].

2. Breast cancer

Breast cancer is the most common cause of mortality in women worldwide. Over 2 million new cases of breast cancer are reported globally [73]. The genetic profile of breast cancer shows high clinical heterogeneity and presence of various molecular subtypes [74]. The complexity of breast cancer is represented by the fact that it comprises a variety of cells, including stem and progenitor cells, instead of a single cell population [75]. Relying on estrogen receptor (ER) expression, the breast epithelial cancer is divided into four subtypes: luminal A, B, triple-negative breast cancer (TNBC), and Her2-positive [76]. Simultaneously, the luminal subtypes are the more fatal and common forms of breast cancer, accounting for around 70% of cases, with 30% of patients resistant to endocrine treatments [77]. Therefore, cytoreductive therapy is critical in the malignancy treatment. In this regard, CRISPR/Cas9 has emerged as a novel and efficient therapeutic tool in the therapy of breast cancer [33]. The knockout of APOBEC3G and CDK4 oncogenes by CRISPR/Cas9 in MCF10A and MDA-MB-231 cell lines, respectively, leads to the inhibition of growth and proliferation of breast cancer cells [78, 79]. On the contrary, knocking down the RLIP and PSMD12 oncogenes in BC and MDA-MB-231 cell lines resulted in decreased breast cell reproduction and development, both *in vitro* and *in vivo* [80, 81].

3. Colorectal cancer

Colorectal cancer (CRC) is a cancer that arises in the rectum and colon, being is the world's ninth most common cancer [73]. Over 90% of all colorectal carcinomas are adenocarcinomas (ADC). Nevertheless, squamous cell, spindle-cell, adenosquamous, and neuroendocrine carcinomas account for the remaining 10% of carcinomas [82]. Mutations in many oncogenes and suppressor genes, including ATF3, NAT1, RBX2, DRD2, and AMPKa1, contribute to colorectal cancer. Thus, the knockout of these genes in the HCT116 cell line by CRISPR/Cas9 could be a promising therapeutic target, and inhibiting them could be useful in the patients with advanced colorectal cancer [83, 84].

4. Liver cancer

Liver cancer is the world's fifth most prevalent cancer and the second leading cause of cancer death, and it is more common in males [73]. Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) are the two major types of liver cancer. HCC and ICC represent 75% and 12-15% of all cases, respectively [85]. However, liver cancer patients have a poor diagnosis and few therapy choices [86]. Therefore, CRISPR/Cas9 technology may be a useful way to find novel therapeutic tools for this malignancy. Various oncogenes, like NCOA5 and Sphk1, were targeted in human HCC cell lines by CRISPR/Cas9 knockout, resulting in decreased cell proliferation, growth, and dissemination, thus reducing tumor development [87, 88]. In contrast, targeting the phosphatase and tension homolog (PTEN) gene *in vitro* by knocking it out promoted the invasion capacity of HCC cells [89]. Hence, with more experiments, CRISPR/Cas9 could have a promising future in fighting hepatocellular cancer.

5. Prostate and bladder cancer

Prostate cancer is the fourth most common cancer-related cause of mortality among males [73]. The prevalence and death rates in PC patients are significantly linked to age, with the peak incidence reported in the elderly (> 65 years) [90]. Prostate cancer is detected by relying on levels of prostate-specific antigen (PSA more than 4 ng/mL), a glycoprotein usually secreted by prostate cells. Albeit, patients without cancer are also found to have high PSA levels. Therefore, tissue biopsy is still the standard method for confirming this type of cancer [90]. Usage of CRISPR/Cas9 to fix the mutations caused by genomic changes might be a promising direction for PC treatment. In particular, it has been found that the knockout of the PTEN gene in PC by CRISPR/Cas9 mobilizes many critical genes for the survival of tumor cells. Moreover, the PTEN cell line showed increased cell proliferation and colony formation [91]. Deficiency of PTEN, a tumor suppressor gene, is associated with the progression, development, and metastasis of prostate cancer. Hence, PTEN knockout by CRISPR/Cas9 *in vivo* could explain the role of many genes with altered expression in PTEN-deficient cells in the development of prostate cancer [91].

On the contrary, bladder cancer accounts for 4.4% of all cancer incidence worldwide, and it is more common in males than in females [73]. Urothelial cell carcinoma causes 90% of all cases, while squamous cells cause the remaining 10% of bladder cancer cases [92]. However, lncRNA UCA1 has

an important role in promoting bladder cancer as an oncogene [93]. In fact, the roles of the UCA1 gene in bladder cancer include increased cell cycle, apoptosis repression, and increased MMP [94]. Therefore, UCA1 knockdown by CRISPR/Cas9 in T24 and 5637 cell lines was shown to reduced cell reproduction, migration, and invasion *in vivo* and *in vitro*. As a result, the cell cycle was arrested at G1 phase, along with significant increase in apoptosis, and decreased MMP activity [93].

6. Cervical and ovarian cancer

Cervical cancer is another common cancer in women, being the third most prevalent cancer among women with a mortality rate of 7.7% [73]. Human papillomavirus (HPV) is among the most common causes of cervical cancer. The HPV produces cervical malignant cells by oncoprotein E7, which inhibits the activity of retinoblastoma family proteins (pRB), and oncoprotein E6, which destructs the tumor suppressor protein p53 [95]. However, the CRISPR/Cas9 technology can destroy HPV E6 and E7, by employing CRISPR-sgRNA to target E7 and E6 *in vitro*. This resulted in reduction in E7 and E6 mRNA and protein expression and accumulation of p21 and p53 proteins. Furthermore, cell growth has slowed and apoptosis has increased, particularly *in vitro* [96].

On the contrary, ovarian cancer is the ninth most frequent malignancy in women and the eighth most fatal among women [73]. About 95% of ovarian cancers are epithelial ovarian malignancies cancers, whereas non-epithelial cancers account for up to 5% of ovarian cancers [97]. In ovarian cancer, the epithelial to mesenchymal transition (EMT) pathway is linked to tumor metastasis, treatment resistance, and a low patient survival rate [98]. Moreover, high expression of the baculoviral IAP repeat containing 5 (BIRC5) gene leads to changes in EMT and tumor growth. Therefore, CRISPR/Cas9-mediated knockout of the BIRC5 gene in SKOV3 and OVCAR3 ovarian cells inhibited EMT, dramatically decreased cell proliferation, and their invasion, prompting cell apoptosis. Hence, in tumors, targeting the overexpressed BIRC5 gene could be an effective anti-cancer therapy [99].

A number of *in vitro* and *in vivo* experimental trials that used the CRISPR/Cas9-based gene knockout technologies in the therapy of various cancers, including lung, breast, colorectal, prostate, liver, and other malignancies are listed in Table 8.

Table 8. Some relevant works on CRISPR applications in potential cancer treatment

Cancer Type	Target Gene	Cell Line	CRISPR effect	Study type	Vector	Action	Gene function	Article
Lung cancer	CD 38	A549	Knockout	*In vitro* & *In vivo*	NV	Decrease cell formation, invasion and metastasis; inhibit tumor growth	OG	[69]
	KRAS	A549	Knock-down	*In vitro* & *In vivo*	AV	Proliferation of cancer cells is reduced.	OG	[70]
	PKP1	SqCLC	Knockout	*In vitro* & *In vivo*	LV	Sufficiently reduce cell reproduction and cell dissemination	TS	[72]
	MFN2	A549 cells	Knockout	*In vitro* & *In vivo*	NV	Enhance colony formation, cell activity, and metastasis by up-regulation of mTORC2/Akt pathway	TS	[71]
Breast cancer	APOBEC3G	MCF10A	Knockout	*In vitro*	Plasmid	Repress cell proliferation	OG	[78]
	RLIP	BC cells	Knock-down	*In vitro* & *In vivo*	LV	Reduce the BC cell proliferation	OG	[80]
	PSMD12	MDA-MB-231	Knock-down	*In vivo*	LV	Decreased development and emigration of breast cells	OG	[81]
	CDK4	MDA-MB 231 cells	Knockout	*In vitro*	Plasmid	Cellular viability, the ability of cells to replicate and growth, and mobility are generally reduced	OG	[79]
	Cdh1	ILC-initiating cells	Knock-in	*In vitro* & *In vivo*	LV	In particular, stimulate *PTEN* gene disruption	TS	[100]
Colorectal	DRD2	HCT116	Knockout	*In vitro*	LV	Decreased activity of anticancer *ONC201*	TS	[83]
	ATF3	HCT116	Knockout	*In vitro*	LV	Decreased invasion and metastasis of tumors	OG	[101]
	AMPKα1	HCT116	Knockout	*In vitro* & *In vivo*	LV	Activating AMPK signaling by targeting PP2A reduces colorectal cancer cells	TS	[102]
	NAT1	HT-29	Knockout	*In vitro*	NV	Under glucose deprivation, increased apoptosis and reduced cell production	OG	[103]
	RBX2	HCT116, HT29	Knockout	*In vitro* & *In vivo*	NV	Decreased cell colony forming and migration	OG	[84]

Cancer Type	Target Gene	Cell Line	CRISPR effect	Study type	Vector	Action	Gene function	Article
Liver cancer	NCOA5	Human HCC cells	Knockout	*In vitro*	LV	Decreased cell proliferation and migration, reduced tumor growth and EMT	OG	[87]
	DEPDC5	Human HCC cells	Knockout	*In vitro* & *In vivo*	LV	Increased cellular ROS resistance, decreased PFS and OS in patients	TS	[104]
	PTEN	Hep3B	Knockout	*In vitro*	LV	Increasing the invasion capacity of Hep3B cells	TS	[89]
	Sphk1	L02 cells	Knockout	*In vitro*	NV	Reduced proliferation and tumor growth of liver cancer	OG	[88]
Prostate cancer	Akt1/2	CWR22rv1 cells	Knockout	*In vitro* & *In vivo*	LV	Decreased metastasis of prostate cancer	OG	[105]
	TP53	PC-3 cells	Knockout	*In vitro*	Plasmid	Reduce cell proliferation and promote apoptosis.	TS	[106]
	PTEN	ΔPTEN	Knockout	*In vivo*	NV	Increased cell growth and colony-forming ability	TS	[91]
Bladder cancer	UCA1	5637 and T24 cells	Knock-down	*In vitro* & *In vivo*	NV	Suppressed cell proliferation, migration and invasion	OG	[93]
	MTHFD2	EJ cells	Knock-down	*In vitro*	NV	Suppressed cell growth, reproduction, and emigration	OG	[107]
Cervical cancer	E6 & E7	SiHa & HeLa cells	Knockout	*In vitro* & *In vivo*	NV	Decreased cell growth and increased cell apoptosis	OG	[96]
	PTEN	EC cells	Knockout	*In vitro* & *In vivo*	LV	Produced PARP/PI3K inhibition in cells, resulting in DNA damage increase and repair defects	TS	[74]
Ovarian cancer	BIRC 5	SKOV3 and OVCAR3 cells	Knockout	*In vitro*	LV	Dramatically decreased cell proliferation, and invasion, and prompted cell apoptosis.	OG	[99]
	miR-21	SKOV3 and OVCAR3 cells	Knock-down	*In vitro*	LV	The EMT in ovarian cancer cells is suppressed	OG	[108]
Thyroid cancer	MFN2	Cal62 and HTH83 cells	Knockout	*In vitro* & *In vivo*	Electro-poration	MFN2 overexpression inhibited EMT, with reduced thyroid cancer cell proliferation and emigration	TS	[109]
	Ku80	K1 and B-CPAP cells	Knock-down	*In vitro*	LV	Excessive apoptosis and decreased growth, invasion, and colony formation	OG	[110]
Melanoma	PTGS2	B16F10 cell	Knock-down	*In vitro* & *In vivo*	NV	Decreased tumor growth and metastasis	OG	[111]
	Pbrm1	B16F10 cell	Knockout	*In vivo*	LV	Increased response to T-cell immunotherapy and decreased resistance	OG	[112]

Abbreviations: AV: Adenovirus, CD8: Cluster of differentiation 38, CDK4: Cyclin-dependent kinase 4, LV: Lentivirus, TS: Tumor suppressor, OG: Oncogene, NV: Not available.

Benefits and disadvantages

In terms of simplicity, flexibility, and low price, the CRISPR/Cas9 system has many benefits over other gene editing technologies like ZFN and TALENs. However, the most significant distinction is that the CRISPR method depends on DNA-RNA recognition instead of DNA-protein interaction [18]. Thus, constructing a customized CRISPR/Cas9 system by simply modifying the guide-RNA (gRNA) sequence rather than designing a novel protein is more feasible and simpler than designing a novel protein [19, 113]. Nevertheless, the huge size of the Cas9 protein is one of the disadvantages of CRISPR-Cas9. Because of Cas9's large size (4-7 kb), it's difficult to pack the protein into low immunogenic AVV vectors used for gene delivery *in vivo* and *in vitro* [114]. Thus, to resolve this issue, the delivery method must be redesigned with a larger cargo capacity, or smaller Cas9 types can be used [115]. Furthermore, clinical trials have shown that Cas9 from *S. aureus* and *S. pyogenes* may cause an immune response within the body [116]. One probable way to override this problem is to upgrade Cas9 or use another bacterial protein that can evade the host's immune system. Another issue with the CRISPR system are the off-target effects that makes it hard to focus on a specific genomic locus [117]. Thus, one of the strategies that may include selection of an appropriate delivery tool that will help to reduce off-target effects while still increasing target performance, such as RNP delivery [118].

Conclusion and future directions

The emergence of the CRISPR/Cas9 system as a bacterial defense response against pathogens, as well as its use as a potent tool for generating selective genomic modifications, has opened new avenues for molecular biology. As an effective editing tool, CRISPR-Cas9 technology has considerable therapeutic potential for improving anticancer approaches, although with certain challenges. Moreover, CRISPR-Cas9 has a wide range of possible applications, including combating oncogenic diseases, modulating gene expression, and immunotherapy. As such, because of CRISPR's medicinal potential, it is regarded as a critical tool in combatting severe cancer disorders. CRISPR is only capable of correcting a single human mutation. However, by driving the technique to its extremes, many genes may be fixed, deleted, substituted, or implanted *in vivo* concurrently with one single strike. Moreover, the development of cas9 forms with no or minimal off-target effects must be considered for future CRISPR uses. Finally, the improvement of non-viral and viral delivery systems will be required to enhance CRISPR/Cas9 *in vivo* application, providing a basis for CRISPR therapeutic use.

Conflict of interest

None declared.

Abbreviations

Abbreviation	Meaning
AAV	Adeno-associated virus
ADC	Adenocarcinomas
AV	Adenovirus
BC	Breast cancer
BIRC5	Baculoviral IAP Repeat Containing 5
C2c1	type V-B CRISPR-Cas system
Cas9	(CRISPR)-associated protein-9
CD38	Cluster of differentiation 38
CDK4	Cyclin-dependent kinase 4
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeat
crRNAs	CRISPR RNAs
DSB	Double-strand break
EMT	Mesenchymal transition
HCC	Hepatocellular carcinoma
HCC cell lines	Hepatocellular carcinoma cell lines
HDR	Homology directed repair
HPV	Human papillomavirus
LV	Lentivirus
MMPs	Matrix metalloproteinase
NHEJ	Non-homologous end joining
OG	Oncogene
ORFs	Open reading frames
PAM	Protospacer adjacent motif
pRB	Retinoblastoma family proteins
pre-crRNA	Precursor CRISPR-RNA
PSA	Prostate-Specific Antigen
PTEN	Phosphatase and Tension Homolog
RNAi	RNA interference
RV	Retrovirus
sgRNA	A single guide RNA
SpCas9	*Streptococcus pyogenes* Cas9
tracrRNA	Trans-activating crisper RNA
TS	Tumor suppressor
ZFNs	Zinc-finger nucleases

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CRISPR/Cas9 как новая технология терапии рака

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Резюме

Рак является заболеванием, обусловленным в основном, генетическими и эпигенетическими нарушениями. Эти заболевания – одна из ведущих причин смерти в мире и представляет собой крупную социальную и экономическую проблему. Согласно статистическим данным, более 10 миллионов человек погибают от злокачественных опухолей, и ожидается 50%-ное повышение частоты их возникновения в следующие 10 лет, приводя к 15 миллионам смертельных исходов. Единичные или множественные генные мутации, хромосомные аберрации могут вызывать раковые заболевания. Хотя для лечения рака используют многочисленные варианты лечения, они все же недостаточны против этих заболеваний. Поэтому изучается ряд новых стратегий ранней терапии злокачественных опухолей. Одной из наиболее современных и потенциально эффективных технологий, применяемых в последние годы для генных модификаций и онкотерапии является система Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-ассоциированного протеина-9 (Cas9) – уникальная технология геномной инженерии, основанная на применении уникальной РНК-содержащей эндонуклеазы. Исходно, CRISPR/Cas9 возникла из противовирусного механизма защиты бактерий от вирусных инфекций. В настоящее время этот подход оказался полезным в лечении рака и генном редактировании. В целом, это сообщение является обзором этой ключевой технологии и ее компонентов. В частности, в этой работе мы касаемся возможных перспективных приложений и нынешних прорывов в технологии CRISPR/Cas9 для лечения рака, а также тех проблем, которые могут возникнуть при клинических исследованиях. В этом отношении мы намерены сделать вклад в оптимизацию работ по CRISPR/Cas9, а также сделать акцент на возможные будущие пути развития этой технологии.

Ключевые слова

CRISP, Cas9, современная технология, рак, терапия.