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CRISPR Cas9: Making Progress Against Cancer

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Abstract: CRISPR/Cas9 (clustered regularly interspaced short palindromic repeat) is one of the most potent methods to genome editing for different organisms. As part of the adaptive immune system of bacteria, CRISPR/Cas9 and its modified versions are used globally in genome engineering to stimulate or suppress the expression of the genes. CRISPR/Cas9 also promises to revolutionize cancer research as it is an efficient technique to get the insights of tumorigenesis, identify developmental drug targets, and arm cell therapies. Currently used applications of CRISPR/Cas9 technology for cancer therapy are reviewed here. In this review, we have enlisted and discussed the impact of CRISPR/Cas9 in creating organoid and mouse models of cancer. Moreover, this review also describes CRISPR Cas9 versatility, in vivo delivery system, drug efficacy, different CRISPR systems, in vivo genome editing, and target discovery.

Keywords: Versatility, cancer model, target delivery, industrial perspectives, innate immunity, RNA interference, gene therapy.

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1. Introduction

With the ever-increasing incident rate, cancer is one of the leading causes of disease-affiliated mortality and global health burden (Alderton, 2020; Biswas and Acharyya. 2020. Rebbeck, 2020; Torre et al., 2012). Scientific advancements and progress have ensured the prevention, diagnosis, and treatment of different cancer types, resulting in extended survival and cure (Lam et al., 2019; Stoffel and Carethers. 2020; Wang et al., 2019). A central pillar in cancer therapy is the improved understanding of underlying tumour and cell biology. This vital knowledge has led to the discovery of small molecules and primary antibody-targeting proteins of oncogenic signalling directions (Kapałczyńska et al., 2018; Larijani et al., 2019; Tower et al., 2020), including imatinib based targeting of BCR-ABL (breakpoint cluster region, Abelson murine leukaemia) in chronic myeloid leukaemia or EGFR (epidermal growth factor receptor) by particular antibodies in colorectal cancer (Cui et al., 2019; Kantarjian et al., 2002; Pottier et al., 2020). Although such agents have improved the survival rate of respective cancer entities. However, for some types of cancer, treatment and cure opinions are within range, and modes of action are poorly detected. Thus, the scientific community considered to characterize the genetic flowchart of cancer and acquire a keen understanding of its role in carcinogenesis and its treatment (Cunningham et al., 2004). Genetic alterations, either particular to a specific cancer type or common to different cancer classes, are revealed through large-scale sequencing projects. Although most of the genetic variations of carcinogenic genomes are well-studied, quite limited is known about the function of numerous mutated genes (Garraway et al., 2013).

The conventional techniques for a detailed and systematic functional analysis of normal or mutant genes are tedious and difficult. Phenotypic variation caused by mutations is either due to random mutagenesis or RNAi associated indirect perturbation of transcripts. Discovery and formation of engineered

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nucleases such as zinc-finger nucleases, also known as TALENs (transcription activator-like effector nucleases), enabled the man to think of directly targeting and modifying the genomic sequence (Prashant, 2013). Lately, DNA engineering showed immensely changed due to the development of CRISPR/Cas9 (CRISPR-associated protein 9) technologies. Since 2013, when it was used for the first time as a tool for editing genes in mammals, CRISPR/Cas9 has significantly developed and spread, allowing the amendments in the genome sequences, the introduction of epigenetics, and transcriptional alterations (Cong et al., 2013). This review is an effort to elaborate on how CRISPR/Cas9 unfolds more horizons for cancer research and its applications as an efficient method, especially in functional cancer genes. Furthermore, we outlined the potential implications of CRISPR/Cas9 to investigate the non-coding carcinogenic genes and try to comprehend CRISPR/Cas9 engineered novel in-vivo cancer models, along with different CRISPR systems.

CRISPR/Cas9 associated gene knockout in Mia PaCa2 cell lines caused the excretion of extracellular vesicles (Pessolano et al., 2018; Belvedere et al., 2016). The knockout of GALNT3 (polypeptide N-acetyl galactosaminyl transferase 3) in Capan1 cell lines observed that some tumorspheres appeared, lacking the ability of motility and regeneration (Barkeer et al., 2018). In another investigation, SphK1 (sphingosine kinase1) gene knockout caused enhanced proliferation and movement (Yuza et al., 2018). Some researchers recently used the same strategy to knockout particular genes in the pancreatic cancer cell lines to analyze various phenotypes (Abdalla et al., 2019; Hwang et al., 2019).

1.1. CRISPR-Cas9

Clustered regularly interspaced short palindromic repeats (CRISPR) express DNA sequences in bacteria and some other microbial sources. CRISPR-Cas9 is a technique that includes the addition or deletion of genes, which made extensive development due to its cost, efficiency, easiness and speed (NimkarandAnand. 2020; Sioud, 2020). The genes which are associated next to these sequences are called CRISPR-associated genes. These genes develop immunity against viruses. There are three primary functions of the CRISPR system: it identifies, cuts, and destroys external DNA. Among the five CRISPR systems, CRISPR-Cas9 is widely discussed. Scientists improved CRISPR-Cas9 to make it useful for the editing of plants, animals, and microorganism's genome (John Travis, 2015). CRISPR/Cas9 is also used to discover gene function in cancer initiation and propagation, gene function in uncharacterized cells and animal models (Ma et al., 2017; Li et al., 2019).

1.2. Gene Editing

There are two main conventional techniques in which radiation and chemicals were used for gene alteration for a specific purpose.

In the 1970s, researchers were able to express foreign genes inserted in an organism's genome from discovering recombinant DNA technology. CRISPR-CAS9 makes it possible to create desired changes, such as insertion, truncation, and modification in an organism's genome. Traditionally, gene editing technologies were able to insert, delete, or modify a single gene; however, CRISPR-Cas9 can perform genome editing, dealing with multiple genes simultaneously (Makarova, 2015). This gene-editing tool is widely used in cancer biology to explore new insights about all types of cancer. In a research study on pancreatic cancer C1GALT1 (core 1 synthase glycoprotein-N- acetyl galactosamine 3-beta-galactosyltransferase1) gene was taken off with the help of CRISPR/Cas9 tool where cells observed to show enhanced growth, tumorigenesis, movement, with the expression of Tn and sTn (Chugh et al., 2018).

1.3. CRISPR-Cas9 Technology mechanism

CRISPR-Cas9 consists of guide RNA and an enzyme that cleaves the DNA. The function of guide RNA targets a specific site on DNA molecule to identify those regions for the break down by the Cas9 enzyme. These broken parts are truncated, inserted, modified, or edited by DNA sequences, and subsequently, these remodelled ends are ligated together. The general mode of functioning of the CRISPR-Cas9 technique is given in Fig.1. Scientists can design guide RNA following any segment of DNA sequence, and this ability has diversified the applications of CRISPR-Cas9 in various fields by using microbial, animal, and plant's genome. However, CRISPR-Cas9 may alter specific cell function by knowing the desired gene sequences (Charpentier et al., 2013).

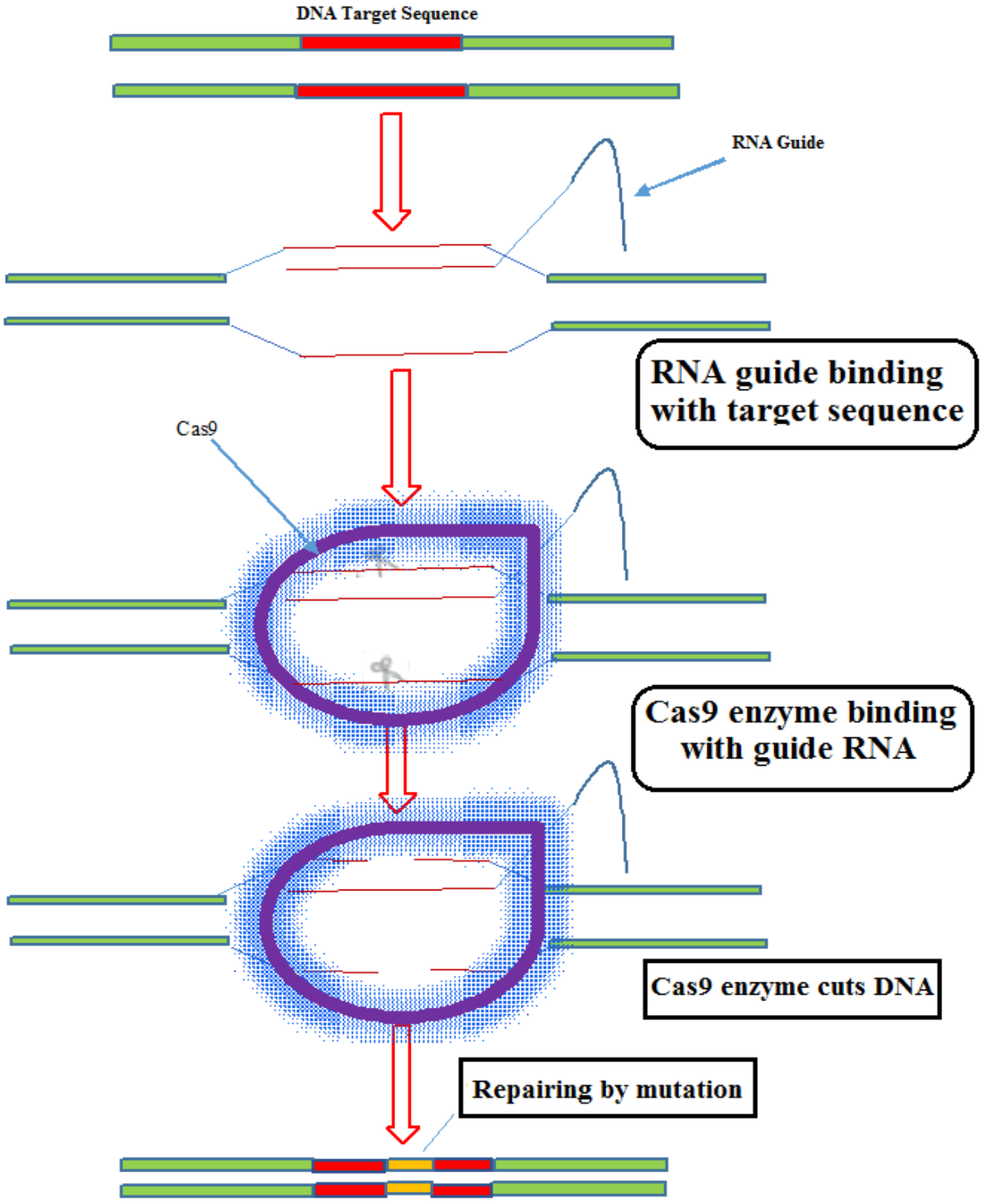

Fig.1. Mechanism of action of CRISPR Cas9 tool

1.4. A Versatile tool for genome remodelling

Clustered regularly interspersed short palindromic repeats (CRISPR) was the first time reported, in the late 1980s, during research on *Escherichia coli* genome (Ishino et al., 1987). Among these palindromic repeats, short spacers (21–72 base pairs (bp)) reported originating from extrachromosomal DNA. Those short spacers may potentially impede plasmid transformation and bacteriophage infection (Barrangou and Horvath. 2017; Jiang and Doudna. 2017).

Table 1. Various vectors and in vivo methods to deliver specific genes encoding Cas9 and sgRNAs to the desired cell

Cas9 system delivery		sgRNA delivery		Invivo delivery	
Encapsulation in viral particle	Lentivirus (Blasco et al., 2014)	Encapsulation in viral particle	Lentivirus (Sanchez-Rivera et al., 2014	Injection of virus or plasmid	Retrograde pancreatic ductal injection (Chiou et al., 2015)
	Adeno-associated virus (AAV)		Adeno-associated virus (AAV) (Platt et al., 2014)		Hydrodynamic tail-vein injection (Xue et al., 2014)
	Adenovirus (Maddalo et al., 2014		Adenovirus (Maddalo et al., 2014)		Intratracheal injection (Platt et al., 2014)
Plasmid DNA	Plasmid DNA (Xue et al., 2014)	Plasmid DNA	Plasmid DNA (Li et al., 2015)		Stereotactic injection (Zuckermann et al., 2015)
Inducible expression in the germline	Doxycycline-inducible expression (Dow et al., 2015)	Expression in the germline	Constitutive expression in the germline (Dow et al., 2015)		Intraductal injection (Annunziato et al., 2016
	Cre-inducible expression (Chiou et al., 2015)			Transfection of plasmid	Plasmid electroporation Maresch et al., 2016)
				Doxycycline treatment	Doxycycline-induced gene expression (Dow et al., 2015)

The purpose of small palindromic sequences was not yet clear. The investigations were expanded to detect the function of CRISPR and Cas genes associated with adaptive immunity towards outsider DNA. Type II system is a subgroup of CRISPR systems that depends on a Cas based protein to cleave the particular DNA arrangement. For the first time in 2013, type II system based Cas protein derived from *Streptococcus pyogenes* applied for DNA lysis in animal cells guided from RNA (Cong et al., 2013; Mali et al., 2013). Before DNA lysis conformation of Cas9 nuclease alters with of sg RNA binding, it is oriented into its specific sequence region (Jinek et al., 2012).

1.5. Different CRISPR systems

Many gene delivery systems for gene encoding have been reported, such as Cas9 system delivery, sgRNA delivery and in vivo delivery (Table 1). CRISPR has two main classes: Class 1 (Type I and type III CRISPR systems, identified in Archaea) and Class 2 (Type II, IV, V, and VI CRISPR systems). Among these various CRISPR/Cas systems of genome targeting, the most commonly used is the CRISPR-Cas9 type II system due to its simple NGG PAM sequence of *S. pyogenes* (Makarova et al., 2011). More than ten CRISPR/Cas proteins were identified recently, like Cpf1 protein from *Acidaminococcus* sp and *Lachnospiraceae* bacterium (Yamano et al., 2016). The Cpf1 needs only one sg RNA (single guide RNA), while Cas9 requires two RNAs. Cas9 is a naturally occurring large protein that may create packaging and delivery issues through adeno associated viruses (Fonfara et al., 2016).

1.6. Target discovery using CRISPR screens

For the development of new targets in cancer treatment, these CRISPR screens are a potent genomic tool. A cell population of different genetic makeup has to be prepared for the screening via CRISPR/Cas9. This CRISPR/Cas9 screening method has many steps in which the first step is the selection of proficient sgRNAs (single strand guided RNAs) for each target gene. Algorithms and bioinformatics tools are used to predict an efficient sgRNAs applied for a specific target gene. After the synthesis of the sgRNA library of oligonucleotides, the lentiviral plasmid is cloned to generate viral particles (Meier et al., 2017). These viruses are subjected to infect Cas9-expressing cells with a slow progression of the infection. From this process, each cell gets a particular gene knockout and a discrete sgRNA cassette. Afterwards, this library of editing cells is placed in a suitable culture medium for a specified duration. Then cells in the culture are harvested for DNA extraction, amplification/extension and sequencing of the sgRNA genes to detect cells having specific gene knockouts (Heigwer et al., 2016). This

simple CRISPR screen procedure was modified by applying various Cas9 variants to get functional screens and evaluate drug-gene interaction. This technique is famous for gene expression with viral vectors into the host cell, along with its complicated and time-consuming procedure (Sharma et al., 2019; Watanabe et al., 2019).

1.7. Identification and investigation over non-coding regions

Genomic DNA having regions of non-proteins coding sequences may consist of enhancers or regulatory elements, or non-coding RNAs contain a vast majority are of the human genome. Most of these kinds of non-coding regions are functionally uncharacterized due to the unavailability of appropriate tools and techniques for experimental performance. In cancer cells, the non-coding RNAs expression is dysregulated or abnormally regulated, while the oncogenes transcription is controlled by the enhancer elements present in the near or distant regions (Datlinger et al., 2017). Thus the comprehensive knowledge regarding the non-coding sequences may give a deep understanding of the cancerous cells. CRISPR Cas9 is identified as the potential tools for the identification and interrogation of the non-coding regions. For the similar purpose, the three cancer-linked genes named as NF1 (neurofibromatosis type 1), NF2 (neurofibromatosis type 2) and CUL3 (cullin-3) genes are screened with the help of CRISPR Cas9 that targets the 700 kb genomic sequence that surrounds the open reading frame of the respective genes (Sanjana et al., 2016). The direct knockout of any individual gene linked to the resistance against the Vemurafenib, a BRAF inhibitor. Furthermore, the downregulation of the gene may phenocopy the disruption of enhancer elements that can achieve drug resistance. Moreover, the alterations in the specific site of the genome upstream and downstream of cullin-3 genes change the dependence of the particular transcription factors by reducing the CUL3 gene product. CRISPR CAS system is used to identify the functional enhancers of TP53 targeted genes (Korkmaz et al., 2016).

The systematic dissection of long non-coding RNAs (lncRNA's) in cancerous cell screening approaches using the three Cas9 variants was performed. Further, Liu et al. (2017) utilized the CRISPRi to screen 16401 lncRNA arrays in the six cell lines for their cell capabilities (Liu et al., 2017). In another similar study, the CRISPRa was used for the lncRNAs identification, which mediates the resistance against the BRAF inhibitions by transcriptional activation (Joung et al., 2017). Many genomic arrays came to know; however, the phenotypic response was caused by the activation of the neighbouring proteins coding sequences. These results opened the new chapter of limitations for the use of the CRISPRa against lncRNAs.

As many lncRNAs are located into the gene coding sequences or have bidirectional promoters, it is infrequent to target the lncRNAs while not affecting the neighbouring genes coding regions (Goyal et al., 2016). Thus, the investigation upon the CRISPR Cas9 technique, which is the most suitable for investigation of lncRNA function is the subject of further studies. In cellular growth, a total of 499 lncRNAs are identified, which are essential for proliferation. Furthermore, the vast majority of the respective lncRNAs are required as cell line-specific manners, which expresses that the lncRNAs expression varies significantly in the cancer spread in different tissues (Zhan et al., 2019).

1.8. Designing of organoid cancer models

Organoids derived from the adult stem cells are becoming popular in the studies of in vitro modelling of diseased and intact human epithelia (Clevers et al., 2016). Stem cells can be isolated from multiple types of adult tissues and cultivated in the three dimensional culturing. These stem cell proliferation, differentiation and formulation into organoids in culture dishes are done by the stimulators, which are tissue-dependent growth factors. The subjective possibility of culturing the cancerous and healthy cells describes the study of the tumour progression in vitro conditions. Schwank et al. (2013) firstly used the CRISPR Cas9 system in the intestinal organoids derived from the mouse body (Schwank et al., 2013). Two years later of this development the two separate groups originate the transformation of cancerous cells from the human colon organoids with the use of adenoma-carcinoma sequences of the cancerous cells of colons with the help of CRISPR Cas9 system (Drost et al., 2015; Matano et al., 2015).

Drost et al. (2017) performed the deletion of DNA repair genes in the colon organoids to model the mismatch repair-deficient colorectal cancerous cells. This helps in the identification of the mutational signature that was present in the cohort's patients of similar defects. Via coupling the scalable culturing and almost intact physiology, organoids are viable for validating findings from the other models in the ex-vivo system (Tao et al., 2016). Furthermore, the new insights can achieve with the help of patents derived

from material data for the functional investigation of compounds or CRISPR screens (Driehuis et al., 2016).

1.9. CRISPR Cas9 in vivo delivery system

An efficient delivery system is required for the delivery of the Cas9 as well as sgRNA to the targeted cell for applying the CRISPR Cas9 in vivo environment. Moreover, the methodology must have high efficiency of genome editing with a low immunogenic effect and capable of directing the sgRNA/Cas9 towards the selected cell or organ type. The first genome editing technique in the mammalian cell was based on the plasmid associated expression of the sgRNA and Cas9 (Cong et al., 2013)**.** In small organisms like rats, this process is useful for the *In vivo* delivery and used as a respective vector may be distributed to tissues with hydrodynamic injections or electroporation (Xue et al., 2014; Maresch et al., 2016). However, for this kind of system, the editing/modifying proficiency is quite low, and the control over the Cas9 activity is inferior. For further improvement in the sgRNA/Cas9 delivery system, several viral or non-viral techniques have been originated. Adeno-associated viruses (or AAVs) are potential tools for the *in vivo* delivery system because it serologically compatible, non-integrating, and have higher transduction efficiency concerning larger fractions of the human population (Luo et al., 2015).

For genome editing, the adeno-associated viruses system may directly be delivered to the targeted organs or systemically administered. Studies performed by Wu et al. explained that sub-retinal injections of AAVs could not efficiently edit the NRL(neural retina-specific leucine zipper) gene in the post-mitotic retinal photo-receptors (Yu et al., 2017). Furthermore, with a similar method, the direct injections of adeno-associated viruses in the mouse striatum can locally modify the huntingtin gene (Yang et al., 2017). For further applications of the adeno-associated viruses, the tissue-based genome editing is successively performed using the specific AAVs serotype tissue-specific promoter for the Cas9. This technique has been effectively used against the dystrophin gene in the muscle tissues (Bengtsson et al., 2017) and the editing of the ornithine trans-carb-amylase gene in the murine liver (Yang et al., 2016). After the particular application, the frequency of editing ranged from 10% to 70%, which is enough for measuring phenotypic improvements in the hereditary disease model of mice (Yang et al., 2016).

The recent studies described that Cas9 mRNA and sgRNA's loading over the lipid nanoparticles and the delivery towards the murine liver contributes the higher efficiency (Jiang et al., 2017; Miller et al., 2017). Moreover, the modified nanoparticles can directly repair the homology. After the intramuscular injection of nanoparticle based-medicine, carrying the donor template sequence, the repair of dystrophin genetic makeup is observed. However, reported efficiency is quite low (Lee et al., 2017).

1.10. Disease models for drug efficacy

Direct administration of a disease-specific drug or medicine in human being before check-in model animal, is not ethical and have social concerns. Several existing models do not match the abnormalities occurred in patients of various diseases. However, it will be costly and time-consuming to meet resembled models with the same conditions of human disease. Currently, CRISPR/Cas9 is widely used to treat anomalies by altering cancer cell lines with good efficacy than conventional procedures, which are less efficient and expensive. A good CRISPR/Cas9 example in which an ID8 (a rat ovarian cancer model) cell line, was genetically edited for the inhibition of BRCA2 (breast cancer type 2) and TP53 that caused a rise in the sensitivity to PARP (Poly ADP ribose polymerase) inhibition (Walton et al., 2016). This rapidly emerging CRISPR/Cas9 speeded up detecting and validating novel drug targets in a specific disease.

1.11. In vivo genome editing

In vivo CRISPR technologies differ from other in-vitro oriented screening methods considering the stronger competition between transplanted cells for growth and survival and several other factors of the microenvironment, such as the host immune system. Chen et al., (2013) completed experiments to recognize genes stimulated development and metastasis through the application of genome-wide CRISPR and keenly studied the non-functional cancerous cells in murine. They transduced a sgRNA library into non-metastatic carcinogenic lung cell lines and transplanted them subcutaneously into mice models. Local tumours were formed by the implanted cells, and lung cells were isolated and evaluated the increased presence of certain single gRNAs. This particular method helped identify stimulators of cancerous cell progression and metastasis(Chen et al., 2013). Besides this experiment, a current attempt was made by Roper et al. (2017) that depicted the possibility of CRISPR based orthotopic inserting of modified colon organoids. It took a few weeks for APC (adenomatous polyposis coli) mutant organoids

to efficiently engraft the colon. Xue et al. (2014) provided the necessary evidence for the first time to support the hypothesis that in vivo gene editing can conclude in tumour formation. They transfected a plasmid having genes and encoding for Cas9 and sgRNAs against PTEN (phosphatase tensin homolog) and TP53 (tumour protein) in liver cells by using tail vein injection. The transfection culminated in the progression of cancerous cells in the murine liver. Afterwards, a similar method was adopted to inject Cas9 encoded specific vectors that targeted a group of 10 genes, leading to the formation of cholangiocellular and myeloma in the animal model having KRAS gene (Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) oncogenic background (Weber et al., 2015). Similarly, plasmids encoding sgRNA are electroporated into the mice's pancreatic cells with a background of oncogenic KRAS(Kirsten rat sarcoma), which causes the formation of tumours (Maresch et al., 2016). Another approach concluded with the glioblastoma development in mice models for six months by applying electroporation of the developing prosencephalon with different tumour suppressors targeting sgRNAs. However, it should be considered that multiplexed sgRNA targeting can culminate in unwanted chromosomal translocations (Maresch et al., 2016). Introducing single gRNAs by an AAVs provides an alternate for such kind of trials which are transfection based. Work of Platt et al. (2014) supported this hypothesis as they introduced AAV, encoding three important Cas9 expressing genes in the lungs. Those genes included single gRNAs directing LKB1, TP53, and KRAS in synergistic combination and a contributor template for KRAS (Platt et al., 2014). In conclusion, macroscopic lung cancer development was observed. Specifically focused AAV-mediated CRISPR genomic library was introduced, after the formation of a visible tumour, into the brain cells of inducible Cas9 expressing mice and targeted sequenced genetic loci (Chow et al., 2017). Frequently mutated sgRNA targeting genes in human glioblastoma also showed extracted lesions enrichment. Moreover, identification of co-occurring sgRNAs suggested a group of combined mutations in glioblastoma. Finally, in vivo and ex vivo administration of single gRNAs and CRISPR-Cas9 may be used to discover new insights into cancer-associated genes' role by exploring the property of their loss-of-function

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2. Environmental issues and cancer

2.1 Cancer and environmental risk factors

Arsenic is a semi-metal chemical element with various allotropes. Its compounds are very harmful that are used in insecticides, pesticides, and herbicides (Tokunaga, 2007). It is known to cause cancer of lungs, skin, liver, bladder and kidney (Abernathy et al., 2003; Rossman et al., 2004; Navarro et al., 2007). Therefore, due to arsenic compounds, millions of people are at cancer and other diseases risk (NRC, 2001; Meliker 2007).

There is a large number of industrial synthetic chemicals that exist in the environment. The amount of these hazardous chemicals considerably increased since the last few decades with the development of science and technology. Pesticides, air pollutants, and lead are among these toxic agents and are serious threats for humans, aquatic, and wildlife. Recently pesticides, cosmetics, and phthalates were detected in surface water and also found in the tissues of fish and shrimps. Some of these toxicants activate estrogen receptors and trigger mammary cell proliferation to initiate the development of the tumour.

Moreover, some heavy metals also have endocrine disturbing chemicals (EDCs) such as cadmium (Cd), which cause breast cancer (Sweeney et al., 2015; Luevano et al., 2014). Many compounds originate from factories and households such as alkylphenols used in detergents, organochlorides used in insecticides and bisphenols, octylphenol, and nonylphenol used in plasticizer's synthesis. The EDCs association of the particular molecules was evaluated in the human and aquatic community (Wang et al., 2017).

2.2. Cancer and pollution

The air quality index is used for the measurement of air pollution. This index can measure six air pollutants such as nitrogen dioxide, sulfur dioxide, carbon monoxide, ozone, suspended particulate smaller than 2.5 μm in aerodynamic diameter, and suspended particulate smaller than 10 μm in aerodynamic diameter (Villeneuve et al., 2014; Hart et al., 2015).

Air pollution is the primary causative agent for the occurrence of cancer and other diseases. Smoking poses a high risk of lung cancer and other respiratory diseases (WHO, 2016). Most of the epidemiological studies evaluated the effect of different types of cigar, cigarette, pipe, environmental tobacco smoking on lungs in which cigarette smoking is the vital threat for

bladder cancer (Stewart et al., 2012). WHO 2014 declared diesel engine exhaust as a carcinogen due to adequate evidence for lung cancer and urinary bladder cancer. The particulate matter in outdoor pollution was classified as a carcinogen for lung and urinary bladder cancer after general population study and environmental levels of exposure (WHO 2016). World Health Organization in 2018 organized a conference on air pollution and set a target to save 70 lac people worldwide from going to death by atmospheric pollution (WHO 2018).

Several types of cancer, especially lung cancer, urinary bladder cancer, and breast cancer, occur globally due to ambient air pollution (AAP). More than 2 million cases of lung and breast cancer were diagnosed, in 2018, to contribute ~11.6% of overall cancer prevalence. Among all types of cancers, lung cancer is the leading cause of death, while breast cancer account 5th in rank. Studies have shown that lung cancer growth closely associated with prolonged exposure to AAP (Hamra et al., 2014; Hamra et al., 2015).

2.3. Industrial prospects and market projections

CRISPR-Cas9 technology has fascinated medicinal industries to show the clinical uses of the CRISPR-Cas9 system in different fields. Several pharmaceutical companies are presently developing the CRISPR-Cas9 system market (Mollanoori et al., 2018), and have revolutionized the therapy of β-thalassemia, sickle cell disease, Leber congenital amaurosis type 10, T-cell mediated immunotherapy in cancer and transthyretin amyloidosis. Based on CRISPR, pharmaceutical companies have invested, in the CAR-T-cell tumour immunotherapy. Bill Gates foundation investing for the progress of CRISPR-Cas9 technology in various fields like livestock, health sciences, malaria and crops (Loria et al., 2018).

Several research foundations have published market scenario of CRISPR-Cas9 and other technologies to emphasize its importance for the development in different fields like livestock, human health, cheeses, research tools, agriculture, medical and many yet to discover (Araldi et al., 2020; Cui et al., 2020; Sioud, 2020; Zhang et al., 2020). In 2017 CRISPR technology market was $477 million, and its estimation by Zion Market Research will get $4.271 billion until 2024. According to the Indian survey report, the world CRISPR market will reach $6.28 billion in 2022 at a compound yearly growth rate (CAGR) of 14.5% (Markets and Markets, 2017). Genome editing market is predicted to expand from $551.2 million (2017) to $3.087 billion (2023) with 33.26% CAGR (Zion Market Research, 2018). A recent report of the North American market indicated the largest share of the CRISPR market due to its advancement and early adaptation for the latest therapy. Subsequently, Asian and European markets were estimated as the second and third-biggest markets for gene-editing technology share (Research and Markets, 2016). A U.S.-based research projection in 2017 proposed that the international market for genome editing will spread to $8.1 billion up to 2025 (Grand View Research, 2017).

3. Future perspectives

Among all gene-editing tools, the CRISPR-Cas9 technique has brought a revolution in molecular biology due to its versatility, advancement, simplicity, and effectiveness in genome manipulation. Along with the widespread use of CRISPR technologies, the most important and effective for therapeutic purposes (Dunbar et al., 2018). Social media and other mass media channels played a crucial in the publicity of these developments in society to create interest in the community about gene-editing techniques. Along with all these quick advancements and improvements in CRISPR-based tools, there are several technical challenges associated with ethical and social concern. One of these difficulties is the transfer of CRISPR techniques into cells and tissues, and scientists use virus-based vectors carrying desired gene sequences. These virus origin vectors cause immunogenicity in living organisms. However, to overcome this problem, AAV vectors are used explicitly as *in vivo* delivery vehicles due to their less antigenic nature.

The main issue is the packaging of Cas9 proteins into the AAV vector due to the bulk size of the Cas9 protein. So it is extremely required to invent a smaller Cas9 protein or truncate its unnecessary regions to decrease its size. Finally, further advancements in the CRISPR Cas9 technique will solve the therapeutic challenges in cancer and many other field challenges. The conventional techniques are not suitable for rapid, efficient, precise, and successful genome editing to cure cancer, infectious diseases, inherited disorders, homologous recombination, and combat with multidrug resistance microbes. I

The Cas 13 RNA screens were used to establish gRNAs for Corona Virus Disease 2019 (COVID-19) infection and for the human RNA segments that may be used in vaccines and diagnostic or treatments (Wessels et al., 2020).

4. Conclusion

Genome editing is a rapidly emerging technology with increased efficiency and low-cost treatment for cancer and other fatal diseases. This tool is making prodigious developments in cancer modelling with precise genomic modifications with good therapeutic value than other tools. Discovery in cancer treatment screens of CRISPR/Cas9 is a useful genomic editing tool for new targets. In both cases, in vivo and ex vivo applications of sgRNAs and Cas9 may be used to discover new insights of cancer-associated genes' function detecting as a loss of function. In future CRISPR Cas9 will deliver an entire group of essential genes for therapeutic purpose and will revolutionize in all fields of life for its novel application at the genome editing level.

List of Abbreviations:AAV: Adeno-associated virus; BCR-ABL: Breakpoint cluster region, Abelson murine leukaemia; CRISPR/Cas9: Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9, CUL3: Cullin-3; EGFR: Epidermal growth factor receptor; KRAS gene: Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; KRAS: Kirsten rat sarcoma; NF1: Neurofibromatosis type 1; NF2: Neurofibromatosis type 2; PI3K: Phosphatidylinositol 3-kinase; PTEN: Phosphatase tensin homolog; TALEN: Transcription activator-like effector nuclease; TP53: Tumour protein; ZFN: Zinc-finger nuclease;

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