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Role of CRISPR-Cas9 in Understanding, Modelling, and Treating Cancer

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ABSTRACT

Background: Cancer is a genetic disease resulting from cumulative genetic/epigenetic aberrations. The genome editing technique known as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 has been widely used for effective gene disruption and modification in a variety of cell types and organisms, both in vitro and in vivo. Cancer treatment using CRISPR-Cas9 has showed a lot of promise. **Objective:** To synthesize current knowledge on the understanding, modeling and treatment of Cancer through CRISPR-Cas9 technique. To delineate about the role of CRISPR-Cas9 in understanding, modeling and treating cancer. Cancer disease, explore the genetic and environmental factors contributing to it, and assess the potential of novel therapeutic approaches like CRISPR-Cas9 towards it. **Methods:** A narrative review was conducted through a structured search of PubMed, Scopus, Web of Science, and Google Scholar, focusing on articles published on role of CRISPR-Cas9 on cancer. Keywords related to CRISPR-Cas9 and its implications across cancer were used to identify relevant studies. Inclusion criteria targeted original research, reviews, and meta-analyses published in English that contributed to CRISPR-CAS9 role in cancer understanding, modeling and treatment. Data extraction and synthesis were performed to highlight key findings, mechanisms, and therapeutic strategies. **Results:** The review emphasizes the critical role of CRISPR-Cas9 in understanding, modeling and treatment of cancer in the wide range. It identifies CRISPR-Cas9 contributing to the treatment of cancer and highlights it has very important role in treating it. Additionally, the review explores emerging therapies. **Conclusion:** The genetic mutations and environmental influences contributing to the cancer and progression. CRISPR-Cas9 has a pivotal role in the treatment of cancer. Understanding the complex mechanisms and working for proper treatment of cancer through it is very essential for devising effective treatment. While significant advances have been made, further research is needed to fully exploit the therapeutic potential of targeting cancer through a gene editing tool like CRISPR-Cas9.

INTRODUCTION

Introduction of CRISPR-Cas9 for Cancer

Cancer world widely found to be the main cause of disease-associated mortality with a high rising incidence (Toree et al., 2012). In Europe, cancer is the third leading cause of death, accounting for 20% of all deaths (WHO, 2021). The progressive accumulation of mutations and epigenetic modifications in the cellular genome causes this deadly illness, which results in persistent growth, resistance to growth suppressors and cell death signals, and a rise in genetic instability throughout the tumorigenesis process. Angiogenesis, invasiveness (metastatic potential), pro-inflammatory activity, and immune system evasion are other crucial characteristics that affects advancement of cancer (Moses et al., 2018). Genetic changes that are either unique to a particular type of cancer or shared by numerous cancer entities have been discovered through extensive sequencing programs. However, a great deal less is known about the function of many altered genes, even though the majority of genetic variants in cancer genomes have been structurally identified (Garraway et al., 2013). It is important to remember that some mutations are unique to particular cancer types and subtypes, offering the foundation for universal or customized treatments that are in line with the genetic makeup of cancerous cells. Traditional cancer treatments include radiation,

chemotherapy, and surgical excision of solid tumors can have poor specificity and numerous, serious adverse effects. With the emergence of hormone treatment, immunotherapy, and targeted medications like growth blockers and anti-angiogenics, oncology trends of today center on improving the safety and selectivity of existing therapies (Cross et al., 2006). In order to permanently alter the genome, gene editing techniques, in theory, use a variety of nucleases to cause single or double-strand breaks (DSBs) to the DNA strand at a precise location. Zinc-finger nucleases (ZFNs) or transcription activator-like effector nucleases (TALENs) served as the foundation for the initial attempts at genetic therapy in oncology (Hazafa et al., 2020). Both approaches use the nuclease FokI to identify specific sequences and use carefully designed protein sets that interact physically with the chosen DNA segment (Zhang et al., 2021). Numerous viruses in the environment pose a threat to prokaryotes' ability to survive (Labrie et al., 2010). Clustered regulatory interspaced short palindromic repeats (CRISPR) are an adaptive immune system that prokaryotes created as a defense mechanism (Ishino et al., 1987). Spacers from bacteriophages and other extrachromosomal components make up a CRISPR locus, which is divided by brief repeating sequences that encode tiny non-messenger RNA. These spacers are adaptive; after a viral attack, bacteria incorporate a new spacer from the phage genome, and the insertion or removal of particular spacers alters the bacteria's resistance to phages. They also prevent infection from their originating viral strains (Barrangou et al., 2007). Furthermore, the CRISPR locus is surrounded by four CRISPR-associated (cas) genes (Jansen et al., 2002). Three phases are involved in CRISPR/Cas-mediated adaptive immunity. According to Sorek et al. (2013), prokaryotes first develop cellular memory of invasive viruses or plasmids. Following infection, an invader's DNA sequence integrates into the host's CRISPR locus as spacer arrays surrounded by repeating sequences (Barrangou et al., 2007), giving the sequence-based phage resistance. Second, RNA polymerase creates pre-CRISPR RNAs, also known as pre-crRNAs, from spacer regions of the CRISPR site (Barrangou et al., 2013). Trans-activating crRNA (tracrRNA) from upstream of the CRISPR locus is transcribed concurrently with pre-crRNA transcription to fulfill two crucial roles: triggering crRNA-guided DNA cleavage and causing the maturation of pre-crRNA of the RNase III enzyme. After then, an active ribonucleoprotein (RNP) complex is formed when the tracrRNA:crRNA complex attaches itself to CRISPR-associated nuclease 9 (Cas9). Third, Cas9 induces a double strand break (DSB) in DNA at a location corresponding to the crRNA spacer sequence due to the double-stranded RNA structure (Jinek et al., 2012). Bacterial resistance to phage infection and plasmid conjugation is a result of the synergistic interaction between CRISPR and Cas9. The advent of CRISPR/Cas9 technology has significantly expedited genome engineering in recent years. Since 2013 (Mali et al., 2013), when it was initially used as a genome editing technique in mammalian cells. The RNA-mediated natural immune defense system (acquired) found in bacteria and archaea is called clustered regularly interspaced short palindromic repeats associated protein 9 (CRISPRCas9) (Wu et al 2014). As its name suggests, its main components are single-guide RNAs (Cr and Cas9 nuclease) (Wong et al., 2015). Double-strand breaks (DSBs) are created at a specific place in the targeted DNA, and it encodes a guide RNA. DSBs are produced when Cas9 nuclease directly binds to a target DNA sequence (Li et al., 2014). In contrast to zinc-finger nucleases (ZFNs) (Wood et al., 2011) and transcription-activator-like effector nucleases (TALENs) (Joung et al., 2013), CRISPR/Cas9 is a facilitator. Single guide RNA (sgRNA), a short complementary strand of RNA, is used by the clustered regularly-interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system to identify and attach to particular DNA sequences via Watson-Crick base pairing. This instructs a Cas9 nuclease to use its two nickase domains, RuvC and HNH, to carry out site-specific DNA cleavage. Compared to ZFNs and TALENs, CRISPR/Cas9 is simpler to program and allows for the "multiplexing" of multiple sgRNAs to target numerous DNA locations at once (Boland et al., 2020).

CRISPR-Cas9 Mechanism: Targeting Genes in Cancer Cells

Delivering its components is a difficulty for CRISPR-Cas9 gene editing. Recombinant retroviral vectors have drawn a lot of attention because of their great precision, specificity, and low off-target consequences when addressing the cell genome. Viral vectors have been employed in genetic engineering for decades. Because Cas9 is big (4.1 KB), lentiviruses have been chosen as an appropriate delivery method (Hazafa et al., 2020). Targeting infectious bacteriophages and invasive viruses, the CRISPR/Cas9 system is a heritable adaptive antiviral immune system of prokaryotes that uses RNA-guided nucleases to break foreign genetic components (Horvath et al., 2010). It has two compartments: one for single-stranded guide RNA (sgRNA) and one for Cas9 endonuclease (Cong et al., 2013). The Cas9 endonuclease is guided by the sgRNA to cleave the target gene's two DNA strands in a sequence-specific way. Three base pairs upstream of a "NGG" protospacer adjacent motif (PAM), DNA cleavage takes place. Following cleavage, the genome's DNA is restored via double-strand break (DNA-DSB) repair mechanisms (Jinek et al., 2012). Thus, by introducing tiny insertions or deletions (indels) through the relatively error-prone non-homologous end-joining (NHEJ) or the high-fidelity homology-directed repair (HDR), the CRISPR/Cas9 gene editing system modifies the genome (Pawelczak et al., 2018). Before introducing programmed genetic modifications, the CRISPR/Cas9 system must reach the targeted cells and pass through the nuclear and cell membrane barriers. Thus far, in vitro administration has primarily relied on straightforward and effective non-specific methods, including electroporation or microinjection, that introduce plasmids expressing Cas9 and sgRNA (Wang et al., 2018). Single-nucleotide alterations in genes such as NOTCH (Misiorek et al., 2021), EGFR (Cheung et al., 2018), and KRAS (Perincheri et al., 2015) account for a significant fraction of genetic mutations in cancer. Base editors (BEs), which are RNA-programmable deaminases linked to CRISPR, may be able to target these point mutations. BEs use a deaminase to enzymatically change the base after

unwinding a brief section of DNA called as the "editing window" at the precise location (Komor et al., 2016).

Identifying Oncogenes and Tumor Suppressors with CRISPR-Cas9

A high-throughput genetic screening method called CRISPR/Cas9 has been used to investigate gene functions and biological pathways connected to cancer (Yin et al., 2019). Cas9 nuclease-mediated loss-of-function mutations are created by introducing a DSB to a constitutively spliced coding exon using specific sgRNAs. DSB site indels, which alter the sgRNA target site and result in gene inactivation, are commonly caused by incomplete repair of NHEJ (Rouet et al., 1994). One of two methods is usually used for cell loss-of-function screening: arrayed or pooled. Since the introduction of oligonucleotide library synthesis techniques, pooled screening has gained popularity because of its advantages, which include low cost and less labor-intensive work. CRISPR/Cas9 pooled screening requires the creation of cell populations with a range of gene knockouts, requiring bioinformatics and several experimental techniques. The sgRNA library is first synthesized into a highly diverse pool of oligonucleotides, which are then cloned into the backbone of the lentiviral plasmid to produce viral particles (Yang et al., 2011). Because virus particles infect Cas9-expressing cells at a low multiplicity of infection during pooled screening, as opposed to array screening, each cell may carry unique sgRNA cassettes and specific gene knockouts. Once these gene-specific knockout cells have undergone precise perturbations, their genomic DNA is collected. The integrated sgRNA cassette is amplified and sequenced to determine the number of cells having those genes deleted in order to monitor the phenotypic impact of those genes. The use of genome-scale sgRNA libraries for gene knockout screening in human or mouse cells demonstrates the potential of the CRISPR/Cas9 system as an efficient loss-of-function screening method and a novel research tool for immuno-oncology (Yamauchi et al., 2018). Here, we report on new discoveries in CRISPR-based target screens for immuno-oncology. One of the primary objectives of CRISPR/Cas9 screening in cancer is to find vulnerabilities unique to an individual's genotype. Targeted deletion of these genes provides a way to find potential therapeutic targets by decreasing the survival of cancer cells (Tzelepis et al., 2016). Finding genes that develop drug resistance or work in tandem with drugs is another application for combining CRISPR screening with drug perturbation, which can aid in understanding the mechanism of cancer response to drug therapy (Wang et al., 2014). Inhibitors of the receptor tyrosine kinase (RTK)/Ras/mitogen-activated protein kinase (MAPK) pathway are used clinically to treat lung cancer and other malignancies; nonetheless, most patients continue to respond poorly to treatment. According to CRISPR/Cas9 gene deletion screening in lung cancer cells, KEAP1 loss changes cell metabolism and allows cell growth without MAPK signaling when many targeted RTK/Ras/MAPK pathway inhibitors are present (Krall et al., 2017). Thus, loss-of-function screening can help determine the optimal treatment plan and evaluate the efficacy of comparable drugs in clinical trials. Neoantigens produced by somatic mutations in carcinogenesis can elicit a robust T cell response; yet, changes can also lead to resistance to immunotherapy. To learn more about how cancer cells avoid being killed by immune cells, sgRNA-transduced cancer cells were cultured with immune cells. The sgRNAs that were enriched or depleted in the cancer cells that survived were subsequently identified using next-generation sequencing. This was done to find the genetic abnormalities that mediate cancer cells' sensitivity or resistance to immune cell death. Loss of important major histocompatibility complex (MHC)-I genes enhances cancer cell escape from T cell death in a co-culture system of human CD8+ T cells and melanoma cells. These crucial genes include HLA-A, B2M, TAP1, TAP2, and TAPBP, according to Patel et al. (2017). Additionally, they are involved in biological pathways such as interferon γ (IFN-γ) signaling, endoplasmic reticulum stress, protein ubiquitination, and EIF2 signaling. Benci et al. (2019) claim that interferon signaling inhibits both innate and adaptive immune killing by acting against immune cells and cancer cells. The efficacy of immune checkpoint blockade (ICB) is directly affected if this connection is broken. Natural killer (NK) cells are crucial for initiating the anti-tumor response. Therefore, identifying specific genes that render tumor cells susceptible to or resistant to NK cell death may result in the identification of novel targets for the strengthening of the NK cell anti-tumor immune response (Cursons et al., 2019). Expression of genes associated with antigen presentation (TAP1, TAP2, and B2M) or IFN-γ signaling (JAK1, JAK2, and IFNGR2) can protect tumor cells from NK cells, whereas JAK1-deficient melanoma cells regulate MHC-I expression by suppressing the IFN-γ-driven transcription events of NK cells, rendering the cells more vulnerable to NK cell-mediated killing. Additionally, tumor cells that are resistant to T cell death become very sensitive to NK cell destruction by selecting clones that lack MHC-I (Freeman et al., 2019). Therefore, NK cell-based immunotherapy may be a strategy to prevent tumor immune escape. Tumor cells that are vulnerable to NK cell-induced cytotoxicity have higher levels of genes controlling chromatin remodeling, lower levels of HLA-E and antigen-presenting genes, and transcription patterns that resemble mesenchymal tissue. An examination of tumor samples from patients receiving or not receiving ICB treatment revealed that the transcriptome linked to NK cell sensitivity is considerably enriched in tumor samples of ICB non-responders (Sheffer et al., 2021). Thus, certain patients are more likely to benefit from NK cell-based therapy than others.

CRISPR-Cas9 in Functional Genomics of Cancer

CRISPR technology plays a crucial role in gene identification, model generation, and resistance control. Its ability to identify and target mutations in cell death signaling pathways makes it a promising option for personalized cancer treatment (Bayat et al., 2024). However, researchers face significant challenges in using CRISPR-Cas9 technology for the efficient targeted delivery of CRISPR components (Liu et al., 2021). The CRISPR-Cas9 editing of the PTEN gene can have a significant impact on cancer progression, as PTEN is responsible for regulating cell proliferation and division. Researchers aim to restore

PTEN's tumor-suppressing function to decrease cancer cell growth and lower tumor development (Cordeiro et al., 2019). In a recent study, Takahashi et al. utilized 3D cancer spheroid models and CRISPR-Cas9 screens to investigate the role of NRF2 hyperactivation in lung cancer growth. They discovered that NRF2 activates numerous oncogenes unrelated to antioxidant activity, including B-cell lymphoma 2 (BCL-2), B-cell Tumor Necrosis Factor α (TNF-α), BCL-xL, Matrix metallopeptidase 9 (MMP-9), and Vascular endothelial growth factor A (VEGF-A) (Zimta et al., 2019). NRF2 hyperactivation is necessary for cell development and survival, while inhibiting ferroptosis helps prevent cell death in matrix-deprived cancer cells (Adamiec et al., 2024). Additionally, researchers identified ferroptosis resistance in hepatocellular carcinoma (HCC) cells through a CRISPR-Cas9 screen, revealing tripartite motif-containing protein 34 (TRIM34) as a key mechanism. Targeting TRIM34 could enhance both ferroptosis sensitivity and the efficiency of immunotherapy in HCC, potentially improving patient prognosis (Adamiec et al., 2024). Another CRISPR activation screen conducted in KP4 lung cancer cells identified BRM as a ferroptosis suppressor, confirming that the SWI/SNF ATPase BRM/SMARCA2 plays a pivotal role in this process (Bhat et al., 2024). Research by Zhang et al. identified TRPML1 as a potential regulator of ferroptosis in AKT-driven cancers. Inactivating TRPML1 or disrupting its interaction with the ARL8B protein inhibits cancer cell growth and increases sensitivity to ferroptosis. Furthermore, a synthetic peptide that targets TRPML1 has been shown to enhance treatment efficacy (Zhang et al., 2024). Another past study revealed that NF-kB, a protein that regulates inflammation, is frequently overstimulated in several forms of cancer (Bonato et al., 2021). CRISPR-Cas9 has the potential to disrupt the NF-kB gene, thereby inhibiting cancer-promoting signaling pathways and suppressing tumor growth. This could enhance cancer cell sensitivity to conventional therapies, potentially reducing cancer progression (Liu et al., 2021).

The Role of Next Generation Sequencing in CRISPR-Cas9

Despite the promising potential for treating diseases, CRISPR/Cas9's safety may be compromised by its dependence on DSBs to initiate the gene editing process. CRISPR/Cas9-mediated DNA breaks have the ability to eliminate thousands of base pairs and produce novel genotypes, some of which may be harmful to cells that are going through mitosis (Kosicki et al., 2018). For example, p53 mutations may result from CRISPR/Cas9 genome engineering in human pluripotent stem cells, which would restrict the use of cell replacement therapy (Ihry et al., 2018). Furthermore, only 38% of HEK293T cells exhibit CRISPR-mediated HDR (Lin et al., 2014), and the high frequency of indel mutations restricts the effectiveness of gene editing (Merkle et al., 2015). However, the majority of recognized genetic illnesses are caused by single nucleotide polymorphisms (SNPs) (Landrum et al., 2016). As a result, techniques are required to precisely alter the sequence of a single base pair at a particular location without adding DSBs. Here, we examine base editors and prime editors, the two newest of these systems. They may be able to get beyond the fundamental drawbacks of conventional Cas9 nuclease gene editing since they are programmable, flexible, and do not require the insertion of DSBs. Nuclease-impaired Cas9 coupled with deaminase is one type of base editor that can induce specific point mutations into DNA without requiring the donor DNA template and HDR or causing DSBs (Rees and others, 2018). DNA base editors come in two main varieties at the moment: adenine base editor (ABE) (Gaudelli et al., 2017) and cytosine base editor (CBE) (Nishida et al., 2016). All four conversion mutations—from C to T, A to G, T to C, and G to A—can be mediated by these two base editors. The few examples of natural deaminases that act on DNA are exclusively efficient on single-stranded DNA (ssDNA), while the majority of known deaminases function on RNA (Harris et al., 2002). When the catalytically damaged Cas nuclease attaches itself to the target DNA strand in CBE and ABE, it partially denaturates the DNA strand that contains the PAM, creating an R-loop that enables the deaminase to carry out an efficient deamination process on ssDNA (Jiang et al., 2017). Base editors are capable of producing the four transition mutations, increasing the effectiveness of point mutation correction, and facilitating the use of gene editing to treat genetic diseases in humans; however, they are unable to produce eight transversion mutations or precisely insert or remove target gene segments (Rees et al., 2018). Furthermore, DNA base editors have the ability to act nonspecifically on RNA and produce single-nucleotide variations, which lowers the specificity of gene editing (Grünewald et al., 2019). The capabilities and applications of genome editing have been significantly expanded by Liu and colleagues' 2019 report on prime editing, which mediates directed insertion, deletion, and all 12 possible base-to-base con versions in human cells without the need for donor DNA templates or DSBs (Anzalone et al., 2019). Prime editors are made up of modified sgRNA, also known as prime editing guide RNA (pegRNA), and an inactivated HNH nuclease (nCas9) coupled to a reverse transcriptase. PegRNA carries new genetic information that can be used as a template to create new DNA strands in addition to binding particular DNA sequences. The Cas9 RuvC nuclease domain nicks the DNA strand carrying PAM when the prime editor first attaches to a particular target DNA sequence under the direction of pegRNA. After the reverse transcriptase reads the RNA and adds matching nucleotides to the end of the nicked DNA, DNA repair machinery stably inserts the new strand into the target site, transferring the modified sequence from the pegRNA to the target DNA. Theoretically, prime editing can fix the majority of genetic abnormalities linked to human genetic disorders, setting the stage for genome editing as a clinical treatment (Anzalone et al., 2019).

CRISPR-Cas9 for Cancer Drug Target Discovery

Since the discovery of CRISPR-Cas9 in 2012 as a gene editing tool, it has been recognized as a significant advancement in drug development (Joy et al., 2023). CRISPR-based screens of cancer cell lines, along with phenotypic assessments like cell proliferation, can help identify genetic connections that may represent potential therapeutic targets. These datasets, combined with other

information, can be used in machine learning models to predict therapeutic impacts on tumor microenvironments better and enhance patient outcomes (Wang et al., 2025). One of the major challenges in cancer treatment is overcoming medication resistance, which complicates treatment effectiveness. Researchers have focused on devising strategies to increase cancer cell sensitivity to therapeutic drugs. Several in vitro methods have been developed to generate drug resistance, mimicking processes observed in clinical settings. These methods include gradually increasing drug doses, extended exposure to fixed drug concentrations, and intermittent treatment cycles. Such approaches facilitate the study of resistance mechanisms and the testing of new strategies to resensitize cancer cells to treatment. ATP-binding cassette (ABC) transporters play a crucial role in drug resistance by actively expelling medications from cancer cells, reducing intracellular drug concentrations and thus undermining their effectiveness. Consequently, efforts to target these transporters to enhance therapeutic effectiveness, using agents such as curcumin, metformin, and other natural compounds are of utmost importance (Vaghari et al., 2020). The CRISPR/Cas9 system, initially discovered as an RNA-guided immune defense mechanism in bacteria, has evolved into a transformative tool for gene editing, particularly for addressing drug resistance mechanisms in cancer cells. Researchers can design specific single guide RNAs (sgRNAs) specific to multidrug resistance (MDR) genes, CRISPR/Cas9 can direct the Cas9 endonuclease to these targeted gene sequences in eukaryotic cells, inducing precise double strand breaks (Hille et al., 2016). This disruption allows for gene deletion or insertion, offering a potential strategy to combat drug resistance.

The delivery of the CRISPR/Cas9 complex can be achieved through various methods, including viral vectors, nanoparticles, or electroporation. Genetic interventions employing this system have shown promising results in increasing cancer cell susceptibility to drugs. Studies targeting the ABCB1 gene a known factor in drug efflux demonstrated that CRISPR/Cas9 mediated knockouts significantly increased the accumulation of chemotherapy agents like doxorubicin (DOX) in cancer cells, thereby enhancing chemosensitivity in resistant cancer types, such as breast and ovarian cancers (Mohammadzadeh et al., 2020). In addition, further research has indicated that targeting genes involved in drug detoxification, such as GSTO1, can enhance chemotherapy cytotoxicity in colorectal cancer, improving the efficacy of drugs like cisplatin. Similarly, disrupting genes related to DNA repair and cell cycle regulation, such as CDK6 and CDK11, has been shown to increased sensitivity to treatments by interfering with cancer cell survival mechanisms. Notably, researchers have demonstrated that CRISPR/Cas9 mediated knockout of CDK6 over resistance in breast cancer cells (Wang et al., 2017). The system also holds promise for treating cancers with BRCA1 mutations. By targeting the synthetic lethality partner of BRCA1, PARP1, with CRISPR/Cas9, researchers aim to enhance the effectiveness of chemotherapeutic agents at lower doses (Wang et al., 2017). Furthermore, investigations into K-Ras mutations in colorectal cancer cells have revealed that targeting these mutations can increase programmed cell death, or apoptosis, when combined with cetuximab treatment. In lung cancer and glioblastoma, targeting mutant forms of the EGFR receptor with CRISPR/Cas9 has led to significant reductions in tumor size and improved survival rates in animal models, showcasing the promising future of this gene editing technology in oncology (Cornell et al., 2019).

Therapeutic Applications of CRISPR in Cancer

CRISPR/Cas9 has had a major impact on gene therapy and molecular biology in recent years. The most prevalent kind of tumors are solid ones, however compared to nonsolid cancers like leukemia, less progress has been achieved in treating them with gene therapy. However, with to advancements in CRISPR/Cas9, this is quickly changing. A rising body of encouraging preclinical evidence suggests that CRISPR/Cas9 is a useful tool for selectively targeting cancer cells and inhibiting the growth of tumors (Floc'h et al., 2018). CRISPR/Cas9 must first be encased in a delivery capsule, such as vesicles, which can be viral or non-viral, in order to guarantee the safety and efficacy of the treatment intended for cancer patients. The CRISPR/Cas9 tools can either generate the complex intracellularly or be delivered as a pre-assembled protein-sgRNA complex. Additionally, it can be supplied as mRNA or as genes that encode the sgRNA and Cas9, which need to be expressed later (Chen et al., 2020). CRISPR/Cas9 technology is being used in more and more clinical trials to treat malignancies of various origins. Instead of focusing on a particular gene in the tumor cells, the majority of these trials use genetically modified T cells for cancer immunotherapy (Saber et al., 2017).

Challenges and Limitations of CRISPR-Cas9 in Cancer

CRISPR/Cas9 genome editing is a promising therapeutic approach for cancer treatment. This innovative approach not only shows potential in normalizing tumor cell epigenomes, but it also plays a crucial role in identifying mechanisms of drug resistance, conducting high throughput genetic screening, and performing gene therapy (Yang et al., 2021). Despite its promise, several significant challenges must be overcome for effective clinical application. These challenges include achieving precise targeting and efficient delivery of the Cas9 system components to the right cells, enhancing the low efficiency of homology-directed repair (HDR), and managing diverse immune responses that can arise in the cancer patients. Recent advancements in both viral and non-viral delivery techniques have improved selective targeting, increased cargo capacity, and reduced immune toxicity during in vivo delivery of Cas9 and single guide RNA (sgRNA) (Rasul et al., 2022). Adeno-associated virus (AAV) vectors are currently considered effective viral vectors for gene therapy, but a significant limitation of AAVs is their restricted cargo size which constrains the amount of genetic material they can carry. Consequently, a strategic approach is necessary, whereby the Cas9 system and sgRNA are encoded on separate, independent vectors to circumvent this limitation and enhance therapeutic efficacy (Senís et al., 2014). CSCs are crucial in developing drug resistance and tumor relapse (Prieto et al., 2017). Tumors contain a variety of genetically distinct cancer cells, including cancer stem cells (CSCs), which react

differently to chemotherapy. This diversity contributes to drug resistance by changing the tumor environment from one that suppresses cancer growth to one that encourages it, highlighting the need for personalized treatment strategies (Phi et al., 2018). Moreover, some of the tumor cells reprogram stromal and immune cells to secrete factors like cytokines that promote tumor progression and inhibit cell death (Zhao et al., 2023). Until now, the in vivo use of the CRISPR/Cas9 system has faced challenges, particularly concerning off-target modifications for therapeutic applications. Researchers are actively exploring bioinformatics tools to address these issues and enhance the system's suitability for treating human cancer. They are utilizing various procedures and investigating multiple bioinformatics tools to improve the effectiveness of CRISPR/Cas9 (Lin et al., 2023). However, current bioinformatics tools are limited in their ability to investigate homologous genes and predict epigenetic modifications. Researchers are also developing strategies to minimize off-target effects of the CRISPR/Cas9 system. These strategies include prime editing, improved Cas9 variants, optimized single-guide RNA (sgRNA), and anti-CRISPR proteins. Additionally, efforts are being made to create modified Cas9 variations and novel gene-targeting methods in mammalian cells that produce minimal off-target effects. This approach holds significant potential for treating genetic disorders in the future. Nonetheless, detecting off-target sites in a sensitive and comprehensive manner remains a major challenge in the field of gene editing (Asmamaw et al., 2024).

Ethical and Regulatory Considerations for CRISPR in Cancer Therapy

Despite being a central tool for future gene therapy, many scientists and bioethicists have expressed concerns regarding CRISPR gene therapy. These concerns can be grouped into three prominent categories: issues of autonomy and informed consent, the evaluation of the risk/benefit ratio, and various justice related concerns (Lange et al., 2022). Germline genome editing raises bioethical dilemmas, including the possibility of unintended alterations in the genome, which could have unpredictable consequences. Furthermore, questions arise regarding the process of obtaining informed consent, and the implications for human reproduction (Ayanoğlu et al., 2020). Animal welfare is another important bioethical consideration. Genome editing techniques can lead to off-target mutations, causing adverse side effects or even diseases in animals subjected to these procedures (Ishii et al., 2017). Additionally, this technology raises broader societal concerns about protecting future generations from non-Mendelian (single gene) illnesses, as well as potential social issues such as socioeconomic disparities, personal values, and affordability (D'Souza et al., 2023). To navigate these complex ethical landscapes, it is essential to establish a comprehensive ethical framework grounded in existing laws and policies. This approach can help balance the remarkable potential of CRISPR-Cas9 technology with the moral responsibilities we hold towards individuals, animals, and future generations.

CONCLUSION

In conclusion, while significant strides have been made in understanding the role of CRISPR-Cas9 in treating the cancer, the complexity of cancer pathology demands further investigation. Future research should aim to elucidate the intricate mechanisms underpinning the cancer, explore the full spectrum of CRISPR-Cas9, and develop targeted therapeutic interventions. Such endeavors will requires the techniques in which gene editing tools like CRISPR-Cas9, leveraging advances in genetics, molecular biology, and clinical medicine to forge new pathways in the diagnosis, treatment, and prevention of cancer disease.

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