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REVIEW ARTICLE

CRISPR-CAS 9: Targeting Angiogenesis in Cancer Therapy

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ABSTRACT

Cancer accounts for around ten million mortalities all around the world. Angiogenesis is the major mechanism behind the formation and subsequent proliferation of cancer cells in body. Hence the need for understanding the molecular mechanism behind the development of cancer is are of higher importance. For efficient treatment of cancer several gene editing tools are available but they lack in providing insights about the off-targets associated with the oncogenic genes. In recent times, the use of CRISPR-CAS9 gene editing tool has gained attention in clinical studies because of their multiplexing capacities; focusing on off-target genes. However, the therapeutic use of gene editing in drug discovery has need to explored to a large extent. This review summarizes the use of CRISPR-CAS9 in targeting angiogenesis and possible ways to develop anticancer therapy.

Keywords: *Cancer; Proto-oncogenes; CRISPR-CAS9; Angiogenesis; cancer therapy; molecular scissors*

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INTRODUCTION

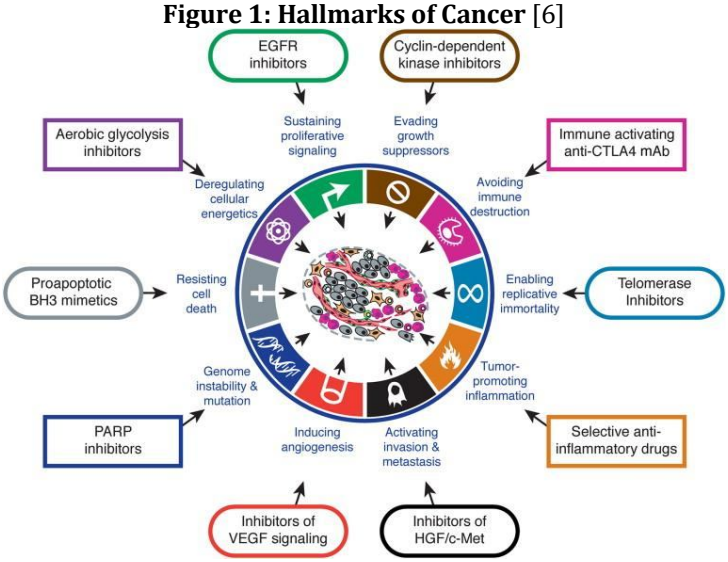
Cancer is a complicated and varied condition mainly due to the dysregulation of epigenetic modifications that drives normal gene expression. Basically cancer is driven on by DNA mutations where oncogenes gets turned on while tumor suppressor genes got turn off [1]. The metabolic deterioration of cancer cells is essential for the initiation and development into tumor cells. In other words, cancer is a cell illness that thrives on alterations in cellular metabolism, their structure, and motility that allows development in unfavorable conditions [2]. The sickness ultimately affects the organism as a whole, co-opting healthy cell types and tissue functions while evading the host's defense mechanisms. The metabolic deterioration of cancer cells is essential for the initiation and development of tumor cell. In order to fulfill the higher cellular energy and metabolic demand as well as reduce oxidative stress, cancer cells autonomously change their flux through various metabolic pathways for their survival in the host cell [3].

Clinical outcomes are influenced by a patient's tumor's molecular characteristics, which may also be utilized in cancer therapy, resulting in more effective medications with low cytotoxicity. Also, environmental nutrient availability and cancer-driver mutations influence the energy flow across these metabolic pathways [4]. Cancer is the second largest disease responsible for causing highest mortality rate. It is a varied illness at the tissue level, which offers a major difficulty for its accurate diagnosis and therapeutic effectiveness [5].

Moving back to the history of cancer Otto Warburg's findings states that cancer tissue slices consumes a significantly higher glucose to produce lactate in vitro, even in the presence of oxygen when compared to

normal cells. His findings states that “damage to the respiration” is a major contributing factor in differentiating normal cells from cancer cells [3].
Six biological characteristics that human tumor acquire during the course of their multi-step evolution constitute the hallmarks of cancer. The distinguishing characteristics serve as an organizing framework for explaining the complexity of neoplastic illness. Cancer cells shows a second level of complexity: they contain a wide variety of recruited, apparently normal cells that aid in the development of distinguishing characteristics by establishing the "tumor microenvironment." The development of innovative strategies to treat human cancer will be increasingly influenced by the understanding of these insights' broad application [6]. The hallmarks of cancer are mentioned in the below:

Figure 1: Hallmarks of Cancer [6]



It is interesting to note that environmental chemicals with carcinogenic tendencies affect cells' cytoplasm and nuclei directly or indirectly, causing gene mutations and genetic alterations. The major contributing factor in the development of carcinogenesis includes: chemical compounds, smoking, viruses, bacteria and radiation rays [7] [8]. Cancer cells alters the cellular interactions and causes critical genes to malfunction. The cell cycle is disrupted by this perturbation, which causes uncontrolled proliferation. During genetic mutation, proto-oncogenes, which are normally responsible for cell development and division, turn into oncogenes, which are the most threatening for cell survival [9].
Currently, there are a variety of highly efficient cancer therapies available. The majority of treatment combine surgery, radiation, and medicine with chemotherapy, targeted therapy, and most recently, home-based immunotherapy. Depends on the what stage of cancer the treatment process will differs. Targeted therapy generally involves the combination of surgery with radiotherapy to diagnose and cure cancer at an early stage [10]. At stage 4 malignancies, the tumor cells have the tendency to spread around the adjacent tissues thus making the treatment more difficult. For such malignancies, strong chemotherapy method is performed which comprises a sustained short-gun defense against these cancer cells [2]. The main drawback is that not every cancer patient will be a good candidate for treatment with targeted therapy.
Angiogenesis is a mechanism in which formation of new blood vessels occurs for the growth and proliferation of tumor cells. Angiogenesis can be controlled by optimizing the production of growth and inhibitory factors in healthy tissues. Proliferating tumors attempt to develop an angiogenic phenotype in order to satisfy their excessive demand for oxygen and nutrients [11][12].
Gene therapy includes modifying DNA or RNA to treat or prevent human diseases. Gene therapy uses a variety of techniques, including correcting, replacing, or eliminating the problematic genes in case of genetic disorders. Conventional genome editing tools inserts the DNA copy into target nucleus but lacks in forming insertional mutations. CRISP-CAS9 system was first identified in bacterial cells as it is a part of their immune defense mechanism against invading viral pathogens [13]. The use of CRISPR-CAS9 as genome editing tool offers several advantages in ease-of-use, multiplexing (numerous genes can be edited at a single time) [14].
Recent review in cancer therapy only highlights the application of conventional genome editing tools but fails to mention about the use of CRISPR-CAS9 editing tool its advantages, mechanism of action and how it

is being significant from standard available genome editing tools. The main objective of the review is (i) to explain the role of CRISPR-CAS9 in targeting tumor angiogenesis (ii) mentioning the advantages of this tool using suitable (iii) challenges in using this tool for cancer drug discovery.

TARGETING ANGIOGENESIS IN TUMOR INHIBITION

Angiogenesis is a multistage process that is activated by a number of biological signals irrespective of whether it has a healthy or pathological consequence [15]. Triggering angiogenesis by the formation of new blood vessels, which is initiated by chemical messengers subsequently causes cancer formation [16]. The most important feature of cancer cells that differentiates from normal cells is that capacity to promote angiogenesis. From the clinical studies it is evident that, targeting angiogenesis will helps in preventing the progression of tumor cells and paves the way for building antiangiogenic drug for cancer therapy [17].

Targeting angiogenic factors will prevents the encircling of blood vessels from proliferation resulting in death of tumor cells due to starvation. Thus, angiogenic therapy focusses on targeting of angiogenic growth factors that leads to tumor cells apoptosis.

Angiogenesis inhibitors are distinct agents in fighting against cancer cells as they prevent the development of tiny blood vessels that promotes tumor growth. The discovery of antiangiogenic drugs, the most of which have concentrated on blocking several metabolic pathways, has resulted in increased advancements in the treatment of cancer [18]. Angiogenesis inhibitors shows best result when used in conjunction with traditional therapies. These inhibitors are administered to patients for a longer period of time as they reduce development of tumor cells without causing damage to adjacent normal cells.

MECHANISM OF CRISPR-CAS9

CRISPR-CAS9 is an adaptive immune system acquired by prokaryotic cells against invading viruses that generally used RNA guide nucleases. The classical genome editing comprises of two major components: one Cas-9 endonuclease and one CRISPR gene that contains single guided RNA or trans-activating crisprRNA (crRNA) [19]. The Cas gene remains in unbound state until bound by the crispr gene sequence. The sgRNA binds with the target DNA and forms a complementary strand against our target of study and acts as scaffold for Cas-9 to come and bind [20]. The seed region is of high target interest since mismatches there might hinder or even totally eliminate DNA binding, but strong homology with this area has been discovered to allow for numerous off-targets with mismatches in other locations. The steps involved in this gene editing are: recognition of active sites; cleavage of DNA sequence; repair mechanism adapted by the host cells [21]. Generally, sgRNA comprises of two major components: the spacer which specifically binds to target DNA. DNA cleavage occurs with the use of “NGG” protospacer adjacent motif that precisely pinpoint the target site. PAM sequence situated three base pairs away from the site this creating the mutation in the area [22]. The breaks in the DNA sequence is repaired by non-homologous end joining and homology-directed repair which are usually error prone repair pathway thus paving the way to study about the particular gene of interest. The major advantage of this technique is double strand DNA break occurs at a sequence specific manner [23].

Mechanism is explained in the following steps:

1. SgRNA binds to Cas-9 which recognizes the target DNA (activation of Cas-9 takes place) via complementary base pairing
2. Once Cas-9 found its target site, it recognizes the PAM sequences causing DNA double strand cleavage
3. HNH cleaves the complementary and RuvC breaks the non-complementary strand leading to the formation of blunt ends
4. Finally, due to error in DNA binding which is attempted by the host machinery, it is useful in studying about the target DNA [24].

Because of its high specificity, higher efficiency, low off-target effects, this editing technique is highly used as unparalleled gene manipulation tool [25]. Unlike ZFM or TALEN the use of RNA nucleases eliminates the need for protein sequences to find multiple gene targets, making it as an easily accessible tool for genetic engineering [26].

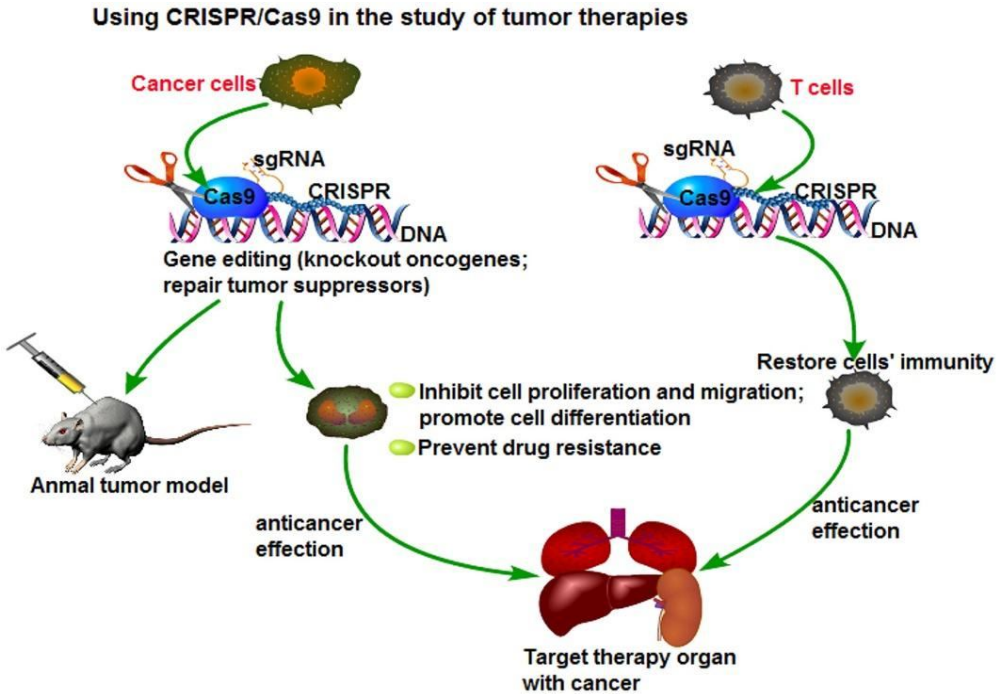


Figure 2: Mechanism of CRISPR-CAS9 in gene editing [21]**.**

For explaining the role of CRISPR CAS9 gene editing tool, here we take TP53 gene which is predominantly found in several cancers but particularly in glioblastoma mulitforme [27]. TP53 mutated gene is most commonly found in half of all cancer cases. Research conducted by American cancer society states that about 32% cancer is caused by TP53 mutation. Mutations in TP53 occurs mainly due to amino acid substitution and frameshift mutation [28]. Proto-oncogenic activation of TP53 results in activation of epigenetic factors which leads to uncontrolled proliferation of tumor cells. One interesting point to note here is not all mutated TP53 is oncogenic. So, the need for therapeutic approaches for gene targeting is highly recommended which can be made possible by use of CRISPR-CAS9 [29].

Several clinical studies on the p53 mutation states that single double strand break caused by CRISPR CAS-9 system is strong enough to cause p53 dependent cell arrest in normal human cells [30]. Report by *Sinha et.al* has arise the question of whether this technique will cause mutations to other genes that are present [31]. From the findings it is evident that Cas9 is responsible for activating p53 pathway resulting in mutation*. Zhanet.al* proposed a p53 sensor that will specifically target mutated version of p53 which will highly eliminates the p53-deficient cells.

Recent computational studies uses statistical framework (CCS) for mapping the DNA fragments which contains mutated genes. Such computational studies provide insights on small number of target region from wide genomic regions [32].

Table 1: Summarizes the available CRISPR-CAS9 for various types of cancer [36]**.**

Cancer type	Target	Cell lines used for study	Clinical Phase Trial	References
Liver	P10	Invitro (mice model)	I/II	[33]
Prostate	P53	PC-3 cell line	I/II	[34]
Lung	P10; EGFR	Invitro animal model	I/II	[35]

APPLICATIONS OF CRISPR-CAS9

Since the CRISPR-Cas9 technology was discovered, potential applications are developing to explore the role in cancer genesis and progression, offering suggestions on how to prevent or treat malignancies that are resistant to treatment [37]. Given this method's flexibility, it has been used to create a variety of genetically modified mice models as well as primary mouse and human cells and numerous cancer cell lines. Chemotherapy involves constant dosages, which worsens treatment-induced toxicity and increases patient costs [38]. CRISPR-CAS9 has the ability to completely damage the genes necessary for tumor cell life which perhaps decrease the cost of treatment.

In an attempt to transfer new scientific techniques from "bench to bedside," CRISPR is quickly advancing toward clinical usage because of the fast improvement of technology [32]. The majority of recent research have pointed to improvements in immune treatments for cancer therapy, where CRISPR altered adaptive cell transfer is a key component [39]. In addition, new DNA and RNA detection methods employing CRISPR-tools have interesting applications in the genotyping and diagnosis of tumor.

LIMITATIONS IN CRISPR-CAS9:

Intense discussions about ethics and the law surround the potential for altering human DNA. Three main concerns have been raised: (i) the potential danger and unpredictability of the technology and its use; (ii) the interference with human germline function and the responsibility to future generations; and (iii) the legalization of human genome editing techniques in light of therapy and enhancement. The development of precise control over long term CRISPR-mediated gene edits via the suppression of Cas9 binding on altered DNA is projected to present both possibilities and problems in recent studies on the arrival of anti-CRISPR proteins.

The emergence of biological weapons is the most terrifying CRISPR-Cas9 system restriction or concern. Through gene editing, bacteria and viruses may be genetically modified to be utilized in biological attacks against individuals or to inflict extensive agricultural damage. The science behind CRISPR have the potential to be used for gene editing, raising major questions about "double-edged sword". The development of novel neurotoxin that end up causing serious disease are just a few of the potentially dangerous biosecurity risks posed by CRISPR-mediated gene editing. As a result, there is a greater potential that may be misused, either by mistake or on purpose by malicious attackers.

FUTURE PROSPECTIVE

CRISPR-based gene modification is still a highly contested topic when it comes to human use. However, after extensive examination, careful thought, and assessment of the risk to benefit ratio, a small number of approvals from FDA resulted in the initiation of a small number of clinical trials for gene therapy utilizing CRISPR [26]. The outcomes of these studies might offer a glimpse into how safe these tools would be in less extreme situations and how quickly they might be put into use as more and more concerns related with the technology are identified and addressed [22]. The greatest fear, however, continues to be that using CRISPR/Cas9 more broadly to treat less severe illnesses might expose the technique to non-therapeutic alterations of the human genome, such as editing embryos to introduce specific aesthetic features in the progeny [40].

More information may be gained about discovering new human diseases due to the research data listed above. In animal models, CRISPR-edited structures for the treatment of a variety of hereditary illnesses, including cancer, eye problems, and thalassemia, have revealed encouraging results. In the near future, it is anticipated that similar possible therapeutic approaches using accurate CRISPR-edited gene constructs would be created to fix the gene mutations of individuals suffering from various human genetic illnesses [41].In the future, the CRISPR technique may potentially be used to resurrect extinct species or perhaps generate entirely new ones. We think that precise gene editing will lead to a bright future for humanity despite all the hazards involved in its deployment [42].

CONCLUSION

The CRISPR/Cas9 gene editing tool has shown considerable promise in research and in the treatment of cancer. This straightforward and adaptable method has the potential to aid in our understanding of the mechanisms behind cancer metastasis, as well as in the foretelling of therapeutic response and drug resistance. Manufacturing cost and timeline, trafficking and tumor infiltration some main challenges in using crispr tool as molecular scissors for gene therapy. To conclude, there are still two major problems that need to be resolved before CRISPR is widely used. The first is how will the therapeutic future of CRISPR be chosen, and who will control the authorizations? And secondly, what moral guidelines ought to be established to make the most of technology while avoiding abuse in order to maximize the benefits of this ground-breaking gene editing method? With the answers to these two issues and the removal of the various restrictions, CRISPR holds the power to advance clinical treatments for human use to a level where miraculous advances in the treatment and management of illness are achievable. Although challenges for CRISPR includes possibility for genomic damage and off-target editing, techniques to assess and reduce such occurrences are already available, and in the end, they are unlikely to materially limit its usage in a therapeutic environment. Many of the fundamental & perplexing issues we have about cancer are already starting to be answered thanks to the fast development and advancements in CRISPR technologies. CRISPR has been, and will continue to be, a crucial tool in our effort to understand and treat

human cancers by defining the role of specific genes in cancer cell lines, enabling the development of next-generation immunotherapies, attributing the functional effect of regulatory elements in tumorigenesis. The need for the development in this technique is higher required as it is still in its preliminary stage, researchers need to develop this technique in point of its efficacy [40].

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