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Review of the CRISPR/Cas9 System on the Development of Novel Cancer-Focused Diagnostics and Therapies

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Abstract

Cancer is one of the most devastating diseases: presenting itself via a variety of hallmark clinical features but a commonality among them is the uncontrolled division and survival of cells due to altered signaling and metabolism. When looking at cancer treatments, patients are given a variety of options such as surgery, chemotherapy, radiation therapy, targeted therapy, immunotherapy, stem cell and bone marrow transplants. CRISPR/Cas9 is a novel genetic engineering technique that enables researchers to precisely manipulate and edit the DNA of living organisms. It is made up of two key components: the Cas9 enzyme acts as a molecular scissor to precisely cut parts of the DNA, and then the guide RNA directs the Cas9 to the specific sequence that it should edit. CRISPR/Cas9 represents a paradigm shift in the field of cancer research and treatment, offering unparalleled precision and adaptability in genetic engineering. The ability to target specific genes and correct mutations has unlocked new avenues for cancer diagnostics and therapeutics.

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1. Introduction

1.1. Cancer

Cancer remains one of the most challenging and life-threatening diseases, appearing in numerous forms and causing millions of deaths each year. Across these types, the unifying characteristic is the uncontrolled growth and persistence of cells driven by disrupted signaling and metabolic pathways.[1] At present, patients can access several forms of treatment, including surgery, chemotherapy, radiation therapy, targeted therapy, immunotherapy, and stem cell or bone marrow transplants. Some of these vary in intensity and duration with some conducted "locally," such as surgery and radiation across a specific tumor or area of the body; while on the other hand, other drug treatments such as chemotherapy, immunotherapy, and targeted therapy can affect the entire body.[2] As cancer-therapy research continues, scientists are looking at CRISPR/Cas9 technology as a novel technique to develop new and improve current therapy options.

1.2. CRISPR/ Cas9

Clustered regularly interspaced short palindromic repeats associated protein 9, otherwise known as CRISPR/Cas9 is a novel genetic engineering technique that enables researchers to precisely manipulate and edit the DNA of living organisms. The technique originates from the natural defense mechanism mediated by RNA that exists within bacteria.[3] To achieve this, CRISPR/Cas9 relies on two key components: a guide RNA to match a desired target gene, and the enzyme, Cas9, which is an endonuclease which causes a double-stranded DNA break, allowing modifications to the genome.[4] The Cas9 enzyme acts as a molecular scissor that is able to precisely cut parts of the DNA, and then the guide RNA is directing the Cas9 to the specific sequence that it should edit.[5]

In this technique, the scientist will first identify the desired sequence of the genome and create a guide RNA that is complementary to this region.[4] Then a Cas9 and the complementary guide RNA are combined into a complex and introduced to the targeted cells; the guide RNA will tell the

Cas9 enzyme to make a precise double-strand break at specific DNA sites.[6] The cell's natural repair mechanisms will eventually fix the break, and this allows for targeted gene modifications.[6] This system's speed, accuracy, and adaptability make it an invaluable tool in genetic engineering.

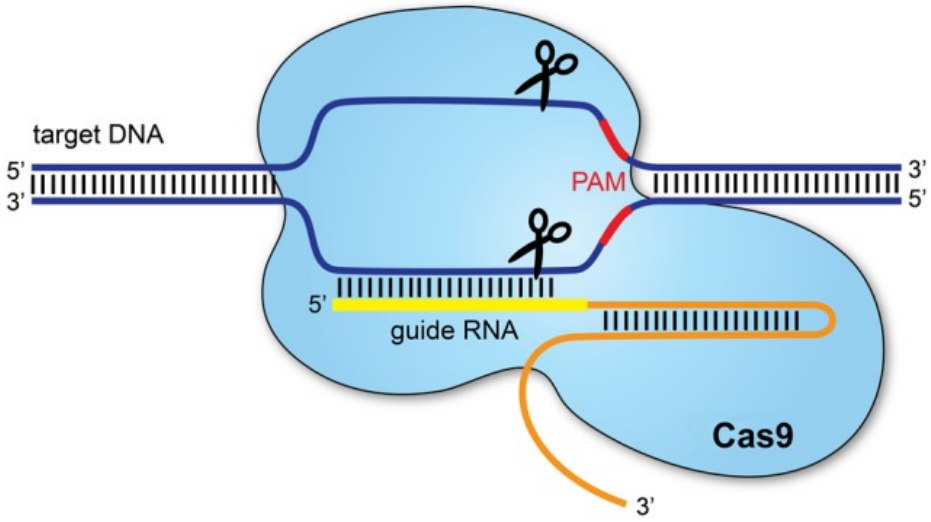

Figure 1: The CRISPR/Cas9 system.[4]

Clustered regularly interspaced palindromic repeats refers to sequences in the bacterial genome and they offer protection against invading viruses when they are combined with a series of different Cas proteins. Cas9, one of the associated proteins, is an endonuclease that cuts both strands of DNA.[4]

With its remarkable potential to develop personalized medicine, CRISPR/Cas9 is poised to revolutionize cancer treatment. This groundbreaking system has already demonstrated success in creating tailored therapies for individual patients in terms of diagnostics and therapies. Its influence within the world of oncology is already vast and significant; yet the highly mutative and adaptive nature of cancer presents challenges and opportunities for further exploration. Continued research is essential in unlocking the full potential of CRISPR/Cas9 in developing future cancer-focused diagnostics and therapies, paving the way for more precise and effective care in combating this complex disease.

2. Genetic Mechanism of Cancer

2.1. Presentation of Cancer

Given that cancer is characterized by uncontrollable cell growth and proliferation, the disease can present itself with a plethora of symptoms and conditions. Whether ranging from asymptomatic to displaying abnormal mass growth to skin lesions, the growth of malignant cells within cancers have many genetic mechanisms.[7] Regardless of the vast symptoms that manifests within cancers, the regulation of oncogenes, tumor-suppressing genes, and self-destruction genes, as well as varying genetic change and mutations are exhibited within each type of cancer.

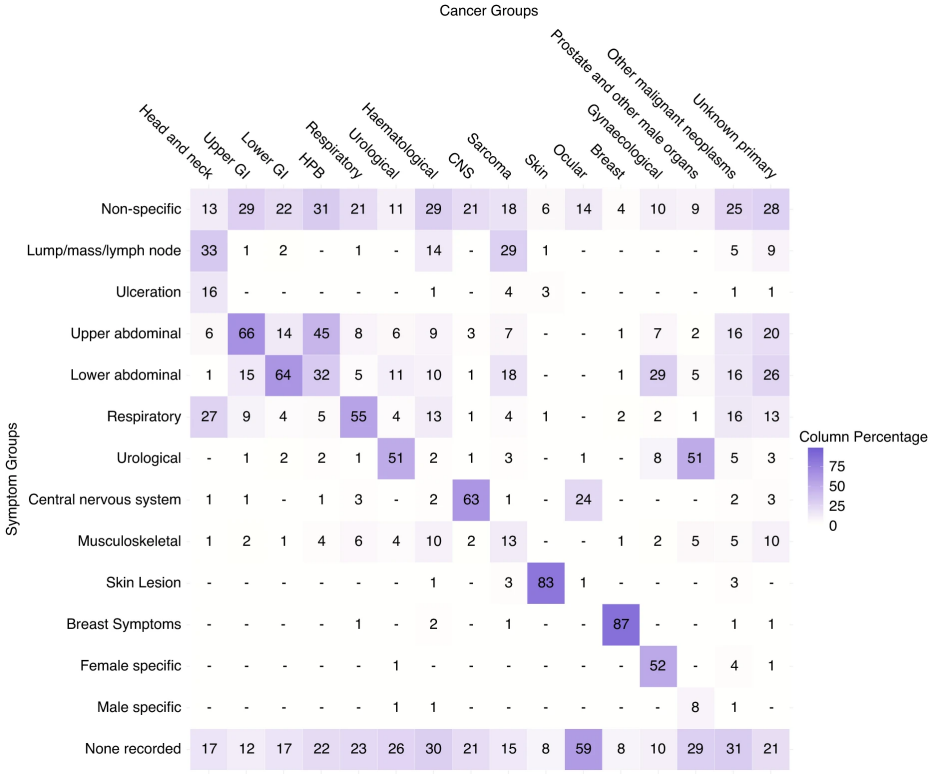

Figure 2: Frequencies of Presenting Cancer Symptoms Amongst Cancer Groups and Cancer Symptoms

2.1.1 Complexity of Cancer

Whereas cancer itself is not hereditary and is not passed down from parent to offspring, factors stemming from genetics can increase one's risk of cancer. [8] On the other hand, mutated BRCA genes, however, can be passed from parents to offspring, and can increase the child's risk of developing breast cancers. [8] Furthermore, the accumulation of the mutations ultimately lead to expression of oncogenes and inactivation of tumor suppressing genes enabling the growth of cancerous cells.[8]

2.1.2. Oncogenes

Proto-oncogenes are critical in maintaining bodily function and regulation through their ability to transmit signals leading to growth factors, allowing for proper cell growth and proliferation.[8] These processes are necessary for human development and growth through basic regulation. Proto-oncogenes are considered "normal" cells that are typically dominant in nature, however they possess the potential to mutate and become an oncogene due to chromosomal translocation, point mutation, and gene amplification.[9] Once transforming into an oncogene, they are incorporated within the genome.

There are currently 50 to 60 recognized oncogenes, many of which are involved in embryogenesis and turned off once the developmental process is completed. [9] However, if turned on afterwards, can lead to cancer.[9] Given that mutations in oncogenes are generally dominant, these epigenetic changes facilitate increased expression of cancerous cells. [9]

2.1.3. Tumor Suppressor Genes

On the other hand, tumor suppressor genes play the essential role in preventing damaged cells from transforming into oncogenic cells through regulation of the cell cycle, acting as a brake as a negative regulator within cell growth.[10] Furthermore, instances of cancer development are often associated with a loss of function in these tumor-suppressing genes, enabling the growth of oncogenic cells, leading to uncontrolled oncogenic

cell proliferation. Whereas oncogenes are typically dominant, tumor suppressor genes are typically recessive, meaning that both copies of tumor suppressing genes must be inactivated to give rise to cancerous cells.[11]

2.1.4 Oncogenes and Tumor Suppressor Genes

In animal-derived models in studying brain cancer, CRISPR/Cas9 has served as a practical opportunity within diagnoses and therapies. This comprehension of declined function in tumor suppressing genes and increased activation of proto-oncogenes allows for the development of gene knockout models.[12]

2.2. Additional Risk Factors

Other non-genetic risk factors for cancer include tobacco use, obesity, physical inactivity, diet, viruses & infections.[13] Combined with genetic factors such as oncogene and tumor-suppressing gene mutations, researchers can tie many associations between cancer and its causes. For instance, cancers such as leukemia, and cancers of the head, neck, bronchus, lung, stomach, liver, pancreas, kidney, ureter, cervix, bladder, colon, and rectum are causally linked to smoking.[14] Factors such as alcohol causally link carcinogenic evidence to causing oral, pharyngeal laryngeal, esophageal liver, and female breast cancer. [14]

2.2.1 Burden of Cancers

In addition to genetic factors, non-genetic factors play a prominent role in the development of cancer. Given that partaking in healthy lifestyle choices can reduce cancer rates by one third, preventative action such as eliminating tobacco use, maintaining a healthy BMI, and participating in an active lifestyle can alleviate risks, combined with the early detection.[13]

Furthermore, the combination of early detection through CRISPR/Cas9 technologies is essential in mitigating disease through advancements in gene editing technologies. Ultimately, the implementation of this innovative

technology will assist with the detection and diagnosis of this complex illness revolving around both genetic and non-genetic factors.

3. CRISPR/Cas9 in Diagnostics

The application of CRISPR offers great promise within cancer diagnostics, particularly within detection of genetic mutations, allowing for extensive screening processes beyond the capabilities of previous gene editing methods combined with increased speed and accuracy.[15]

As seen within various diagnostic platforms, including DETCTR, DNA endonuclease-targeted CRISPR trans reporter, and SHERLOCK, specific high-sensitivity enzymatic reporter unlocking will enable accelerated diagnosis of cancers when utilizing Cas12 and Cas13 systems. [15]

3.1 CRISPR/Cas9 Functions

CRISPR/Cas9 operates to target and knock-out overexpressed oncogenes responsible for growth of cancerous cells such as MYC, KRAS, and EGFR to ultimately slow cell proliferation.[15] Current diagnosis and treatment reflects the effectiveness of CRISPR in knocking out KRAS to inhibit cancerous colorectal cells. In addition, CRISPR can be integrated within tumor suppressing genes to correct mutations and regulating the cell cycle.[15]

3.1.1 Cancer Drug Treatment Targets

In addition, this novel technology enables future diagnoses and drug development combatting cancerous cell growth. Under the Wellcome Sanger Research Institute, CRISPR/Cas9 has been extensively studied across over 300 types of cancerous cells–by identifying targets for tissues and genotypes, researchers concluded that Werner Syndrome ATP-dependent helicase (WRN) is susceptible as a target in cancerous cells with microsatellite instability.[16] These studies can further propel cancer drug treatment developments through generating new targets of higher specificity.

4. CRISPR/Cas9 in Therapies

4.1. Novel Treatments

CRISPR/Cas9 has emerged as a groundbreaking tool in enhancing cancer treatments.

4.1.1. Chimeric Antigen Receptor T-Cell Therapy

Cell therapy is an advanced form of treatment where living cells are introduced into a patient's body to repair or replace damaged tissues or cells, or to stimulate the immune system to fight diseases, such as cancer. [17] In particular, Chimeric Antigen Receptor (CAR) T-cells represent a breakthrough in personalized cancer therapy. CAR T-cell therapy is a novel form of immunotherapy that harnesses genetically modified versions of a patient's own T-cells to fight against cancer.[17]

In this therapy, the chimeric antigen receptors that are present on the surface of the newly created CAR T-cell will recognize specific antigens on cancer cells.[17] As these antigens are encountered, the CAR T-cells will activate, causing them to specifically target and kill these cancer cells. The key benefit of CAR T-cells is that they persist in the body long-term and will continuously recognize and attack cancer cells.[18]

T-cells are first collected from the patient's blood through a process called apheresis, which are then sent to a lab where the modification of adding a new gene to the T-cells occurs. In this treatment method, synthetic receptors consisting of antigen recognition, signaling, and costimulatory domains are utilized to reprogram T-cells in order to target tumor cells for destruction.[19] Chimeric antigen receptors are proteins produced by the fusing an antigen-binding domain—typically an antibody-derived single-chain variable fragment (scFv)—with CD3ζ, a T-cell receptor signaling domain.[20] The tumor-specific CAR causes T-cells to act independently of major histocompatibility complex-restriction, and allows any target expressed on the surface of cancer cells to be recognized.[20] Further co-stimulation is given simultaneously with the signaling domains of molecules

like CD28 or 4-1BB to further potentiate T-cell activation and persistence.[19] After the signal is recognized, the T-cell-intrinsic cytotoxic machinery is unleashed: clonal expansion occurs where the T-cells will rapidly multiply into a large population of identical cells thanks to assistance of the cytokine interleukin-2, and upon encountering the target cells, cytotoxic molecules like perforin and granzyme are released to kill them.[21]

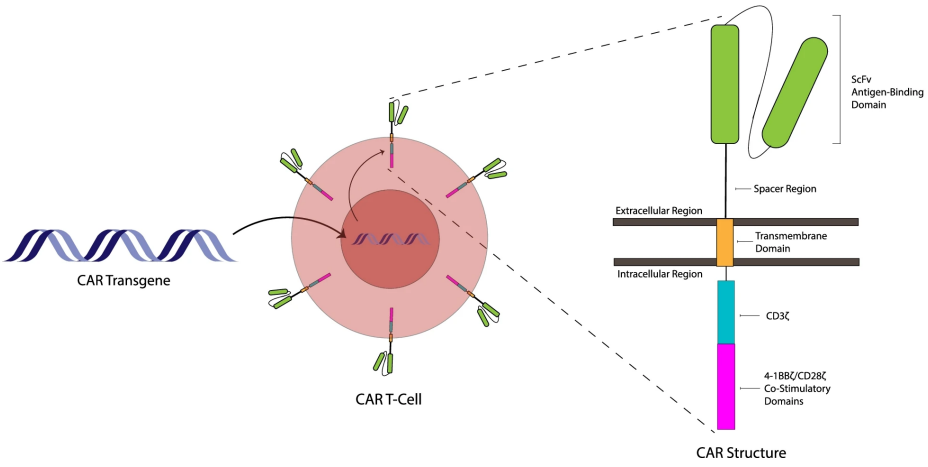

Figure 3: Schematic representation of a chimeric antigen receptor (CAR) T-cell.[19]

Factors such as T-cell exhaustion, lack of CAR T-cell persistence, cytokine-related toxicities, and bottlenecks in the manufacturing of autologous products have hampered the safety, effectiveness, and availability of this approach.[19] The potential of CRISPR/Cas9, however, holds the key to address many of these issues. Focus on precision engineering of CAR T-cells, CRISPR-Cas9 systems can install desired genetic changes with or without introduction of a double-stranded break into the genome.[19] CRISPR/Cas9 has been used to create a memory phenotype in CAR T-cells, this modification further improves their long-term persistence in the body.[22] Additionally, CRISPR/Cas9 enables researchers to screen for new targets to also improve the anti-tumoral potential of CAR T-cells.[22]

4.1.2. Oncolytic Virotherapy

Oncolytic virotherapy is an innovative cancer treatment approach that relies on modified viruses to target and destroy cancer cells while sparing normal cells. [23] These oncolytic viruses are designed to selectively infect, replicate within, and kill tumor cells, leading to direct cancer cell destruction and an immune response against the cancer.[23] This therapy exploits the vulnerabilities of cancer cells to optimize cancer cell death: direct tumor destruction and the activation of systemic anti-tumoral immunity.[23]

Oncolytic viruses work via two main pathways: directly attacking the cancer cells and inducing an immune response.[24] In the first method, the viruses are engineered to propagate selectively in tumor cells; this is to specific characteristics like defective antiviral responses and altered metabolic states.[24] Once infected, as the virus continuously replicates in the tumor cell, it causes the infected cell to burst, leading to cell death while also releasing viral particles and tumor antigens in a process called oncolysis. In this part, there is minimal damage caused to normal non-cancerous cells.[25] Then the particles and antigen released causes an immune response which results in the recruitment and activation of immune T-cells—this immune response targets not only the original tumor cells but also distant metastases.[25]

Commonly, to produce these virus, there are three main methods: via the homologous recombination system based on bacteria, the bacterial artificial chromosome system, or the hybrid yeast-bacteria cloning system.[26] In a typical scenario, to create an oncolytic virus, researchers will start off with a base virus—adenoviruses, herpes simplex virus, vaccinia virus, reovirus, etc., the following steps will differ slightly depending on the virus.[27] Though generally, it will involve the deletion or mutation of the genes that promote viral replication in normal cells but not cancer cells and in doing so this can enhance tumor selectivity. [27] For example, within adenoviruses, the genes, E1A and E1B, inactivate pRb and p53 in normal cells, respectively, to delay premature, programmed cell death, but by deleting the E1 gene, the virus is made tumor specific.[28] Transgenes encoding immunostimulatory molecules such as GM-CSF, IL-12 may be inserted to further boost immune responses. [26] To achieve this, CRISPR/Cas9 can be utilized to manipulate

the genome of the virus—mutating the E1B gene to prevent replication in cells with functional p53, enhancing tumor selectivity.[26] The oncolytic virus can then be injected into the patient, at which the virus will do its intended job.

In oncolytic virotherapy, CRISPR/Cas9 is critical in revolutionizing its development by ensuring precise genetic engineering. Beyond the ability of deleting and modifying particular genes, CRISPR/Cas9 technology is key in improving safety and the development of combined therapies.[12] For example, unwanted side effects can be minimized by using CRISPR/Cas9 to edit out viral genes responsible for pathogenicity in normal cells.[29] Additionally, CRISPR technology is able to develop oncolytic viruses that have the capabilities of complementing pre-existing cancer immunotherapies.

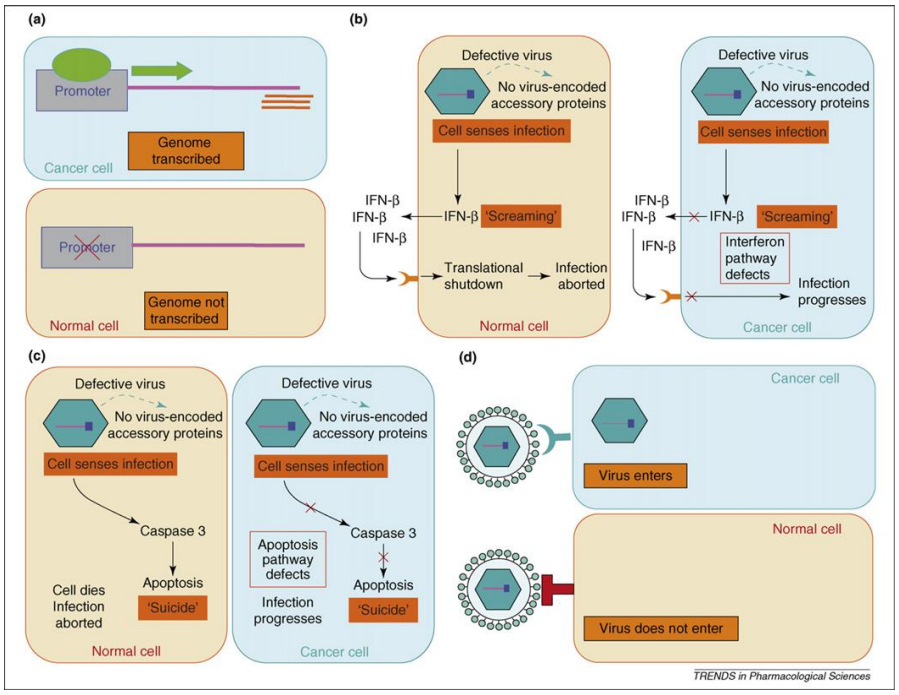

Figure 4: Mechanisms of tumor targeting by oncolytic viruses. *(a) Transcriptional targeting. (b) Translational targeting. (c) Pro-apoptotic targeting. (d) Transductional targeting.*[25]

4.1.3. Macrophage-Based Therapy

Macrophage-based therapy is an emerging immunotherapy approach that employs the versatile functions of macrophages in the goal of treating cancer. The technique leverages macrophages, which are innate immune cells, due to their dual role in cancer progression: capable of promoting and suppressing tumor growth.[30, 31] In particular, due to their functional plasticity and capacity to infiltrate tumors, they are ideal candidates for cancer-focused therapies.[32] Additionally, by leveraging the advances and innovations made in genetic engineering—CRISPR/Cas9 technology, researchers have made incredible growth in the progression of macrophage based therapies further enhancing the antitumor capabilities of these macrophages.

Macrophages in tumors can adopt either a pro-tumoral (M2) or anti-tumoral (M1) phenotype; these macrophages are known as tumor-associated macrophages (TAM). M1 macrophages, when activated, will exhibit pro-inflammatory, cytotoxic properties that directly targets and attacks tumor cells.[33] Additionally, they activate an adaptive immune response, which produces reactive oxygen species, tumor necrosis factor-alpha, and interleukin-12, which enhances immune responses and inhibits any further growth of these tumors. [34] In contrast to the anti-tumoral phenotype, the M2 macrophages promote tissue repairs, angiogenesis, and immunosuppression through the secretion of anti-inflammatory cytokines like interleukin-10 and transforming growth factor-beta—supporting the suppression of immune activity and growth progression of tumors.[33]

The goal of the macrophage-based therapy is to reprogram or recruit macrophages to favor the anti-tumoral (M1) phenotype along with enhancing the tumoricidal properties. [31] The development of macrophage-based therapies involves several complex steps. They first need to be isolated from the patient's blood or bone marrow and grown via ex vivo to produce sufficient quantities for use.[31] CRISPR/Cas9 is then used to improve anti-tumoral properties—precise editing of genes enhance precise and particular macrophage activation, polarization, and immune signaling.[35] By removing genes involved in immunosuppression and inserting genes enhancing tumor

recognition, the macrophage can be optimized for tumoricidal activity.[35] Additionally, CRISPR/Cas9 can be harnessed to alter macrophage surface receptors: eliminating the signal that inhibits phagocytosis, enabling macrophages to efficiently attack tumors.[36] Following the optimization of their anti-tumoral properties, chimeric antigen receptors are engineered into the macrophages to develop chimeric antigen receptor macrophages (CAR-M).[37] Their metabolic pathways are also altered to keep these macrophages in the M1 states, ensuring their sustained activity within the tumor microenvironment.[37]

Macrophage-based therapy represents a promising frontier in cancer treatment, and with the use of CRISPR/Cas9 technology, potential of these therapies are amplified by enabling precise genetic modifications to improve efficacy.

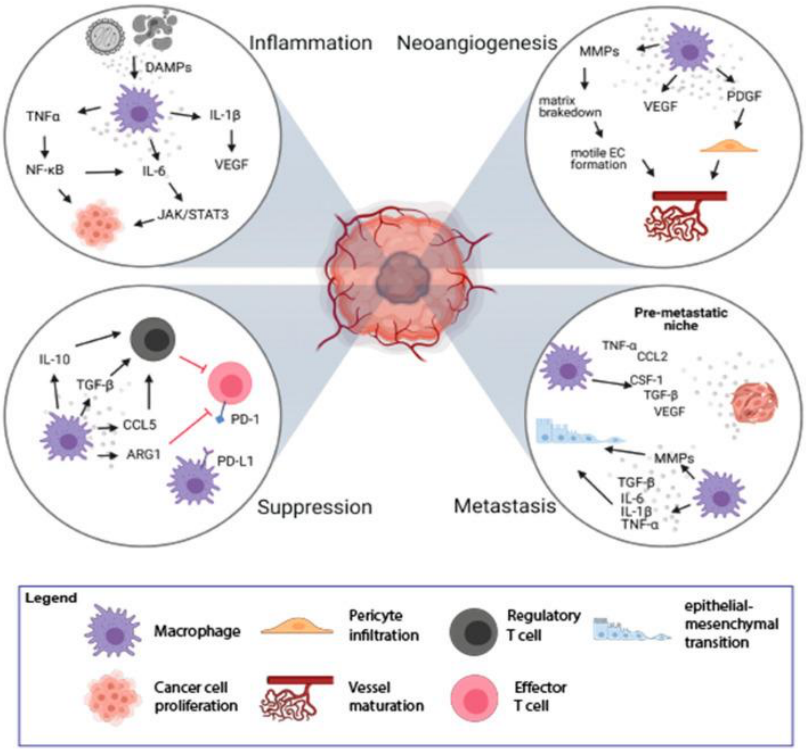

Figure 5: The mechanisms of tumorigenesis stimulation by TAMs.

The illustration shows induction of inflammation (top left loop), stimulation of neoangiogenesis (top right loop), immune suppression (bottom left loop), and induction of metastasis (bottom right loop).[31]

4.2. Pre-Clinical Testing

Pre-clinical research is a critical step in the creation and development of any cancer therapies and that includes any that involve the usage of CRISPR/Cas9 systems. These studies vary widely in their methods, to approaches, to goals; though ultimately, a key goal among them all is to evaluate the safety, efficacy, and potential side effects of a new therapy. The pre-clinical research on the application of CRISPR/Cas9 continues to grow with innovative breakthroughs being made regularly. A pre-clinical study that demonstrates this is Martinez-Lage et al.[38]

4.2.1. Targeting Oncogenic Gene Fusions

Fusion oncogenes are common in many forms of cancers and are chimeric genes that result from in-frame fusions of the coding sequences of two genes in chromosomal rearrangements.[39] They play a role in tumorigenesis, which is the process by which normal cells transform into cancer cells, forming a tumor, by promoting cancer cell growth and survival.[39] Martinez-Lage et al.'s research focuses on targeting oncogenic gene fusions: the selective elimination of cancer cells with fusion oncogenes.[39] In particular, they are harnessing the potential of CRISPR/Cas9 systems. This approach is exploiting the specificity of CRISPR/Cas9, which enables researchers to create double strand breaks in intronic regions.[39] The ultimate goal of this method is to disrupt oncogene expression in such a way causing tumor cell death without affecting normal cells.

This strategy focuses on unique fusion characteristics and has demonstrated potential effectiveness. The study demonstrated that the CRISPR/Cas9-induced genomic deletions were highly specific to the cancer cells with these fusion oncogenes, leading to cell death without any impact to normal cells.[39] Additionally in the in vivo experiments, they demonstrated a high-degree of efficiency in inducing cancer cell death, reducing tumor burden and mortality.[39] Ultimately, this study establishes CRISPR/Cas9 as a potential and effective tool for targeting fusion oncogenes in cancer therapy.

4.3. Clinical Applications

In CAR-T immunotherapy, researchers genetically engineer T cells to have a receptor that recognizes a patient's cancer cells, telling the T cells to attack.[20] Thirty years after the first publications of the chimeric antigen receptor, the U.S. Food and Drug Administration (FDA) has approved the first anti-CD19 CAR T cell therapy.[20] They approved CAR-T made with traditional gene therapy in 2017 and it's proven to be an effective approach.[41]

4.3.1. CRISPR Therapeutics - B-Cell Malignancies

Two examples of ongoing clinical trials involving the use of CRISPR/Cas9 are CRISPR Therapeutics' programs on B-cell malignancies, particularly relapsed or refractory cancers like non-Hodgkin lymphoma and chronic lymphocytic leukemia. [42] They are currently developing an allogeneic CAR-T cell therapy method; the underlying goal is to design a therapy that targets the CD19 protein, which is commonly expressed on the surface of B-cell cancers.[42] The CD19 protein is a well-established target since it is present in various cancers such as non-Hodgkin lymphoma and acute lymphoblastic leukemia.[42] In particular, the program is leveraging CRISPR/Cas9 gene-editing technology to modify T-cells, enhancing their anti-cancer capabilities.[43]

The first generation of this is the CTX110; here, the CRISPR/Cas9 technology is being used to delete the T-cell receptor gene and CD52 gene on T-cells so that these cells do not recognize and attack each other in an immune response.[43] After the modification, these T-cells are placed back into the patient with the goal of killing cancerous B-cells. [43] CTX110 is currently being tested in the CARBON Phase 1 trial, which has shown promising results in terms of safety and preliminary efficacy.[44] The study is ongoing, with a focus on assessing the therapy's dose-dependent safety and efficacy profile.

The next generation is known as CTX112, which incorporates additional gene edits with the goal of enhancing potency and reducing T-cell

exhaustion.[45] In preclinical studies, these edits demonstrated a tenfold improvement in potency compared to first-generation candidates. [45] This product is designed to be more effective against B-cell malignancies by editing multiple genes (TCR, CD52, and PD-1 genes) in the T-cells, including changes aimed at reducing T-cell exhaustion.[45] CTX112 is currently undergoing Phase 1/2 clinical trials to evaluate its safety, tolerability, and effectiveness.[46]

5. Conclusion

CRISPR/Cas9 represents a paradigm shift in the field of cancer research and treatment, offering unparalleled precision and adaptability in genetic engineering. From the application of CRISPR in diagnostics platforms like SHERLOCK and DETCTR to enhancing CAR T-cell therapy, improving oncolytic virotherapy and macrophage-based treatments, CRISPR/Cas9 continues to push the boundaries of cancer diagnostics and treatment.

5.1. Safety Risks and Ethical Concerns

In spite of the novelty of CRISPR, it is equally as important to recognize the safety risks and ethical concerns that surround this technology. Although CRISPR/Cas9 can be very precise in its modification of the genome, the potential to inadvertently edit the unintended parts of the genome is still very possible, and this can lead to potentially very detrimental consequences.[47] Another safety is the matter of mosaicism, an issue that arises with only some cells receiving a genetic edit while others do not, leading to a mixed population of edited and unedited cells.[48] Most critically, it is important to recognize that studies have suggested that CRISPR may also impair cancer-fighting abilities of cells. This means while CRISPR holds so much potential in cancer treatment, there are still many risks involved hence the need for further research in both the pre-clinical and clinical setting to ensure the safety of the therapies being developed.

Ethical concerns are raised constantly regarding the usage of CRISPR/Cas9. In particular, the modifications of genes in embryos that could be passed onto future generations raises significant ethical questions

regarding safety.[49] Additionally, there is immense worry about the potential misuse of CRISPR for non-therapeutic enhancements or selective breeding, leading to ethical issues of eugenics.[50] Furthermore, there are challenges regarding the ability to obtain a truly informed consent from a patient, especially for future generations as CRISPR technology becomes more complex.[50] There are also concerns that CRISPR-based therapies can exacerbate existing healthcare disparities, becoming an option that is only available to the wealthy.

5.2. Future of CRISPR

The future of CRISPR is immensely promising as it continues to revolutionize medicine beyond the scope of cancer. In general, CRISPR holds immense potential for treating genetic diseases by correcting mutations at their source. Researchers are actively and continuously exploring its application in gene therapies for other conditions like sickle cell anemia and cystic fibrosis.[51] Additionally, scientists are refining delivery methods, such as lipid nanoparticles and viral vectors, to ensure accurate targeting and minimize off-target effects.[52]

Beyond healthcare, CRISPR is expected to transform the world of agriculture by creating crops that are more resistant to pests, diseases, and environmental stresses, while also improving yield and nutritional value.[53] Livestock breeding could also immensely benefit from gene editing to enhance desirable traits, promoting sustainable food production.[54] In synthetic biology, researchers are looking to design gene circuits for bioengineering applications including biofuel production and environmental cleanup through engineered microorganisms.[55] Ultimately, the future of CRISPR promises groundbreaking solutions to some of humanity's most pressing challenges, redefining the boundaries of genetic engineering and personalized medicine.

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