Targeting Epigenetic Modifications in Benzene-Induced AML with CAR T-Cell Therapy and CRISPR-Cas9

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ABSTRACT

While the connection between genes and cancer development may be well-defined, the link between environmental exposures and the emergence of hematological malignancies remains ambiguous. This research paper explores the converging domains of benzene exposure and epigenetic modifications as pivotal contributors to the pathogenesis of Acute Myeloid Leukemia (AML). In particular, DNA methylation has emerged as a key factor in the translation of benzene exposure into abnormal gene expression profiles and disrupted hematopoietic differentiation. This review will also provide an in-depth analysis of challenges associated with existing CAR T-cell therapy for AML treatment and propose a groundbreaking alternative: CRISPR-Cas9. This innovative tool has proven to deliver enhanced effectiveness, precision, and versatility in addressing certain epigenetic modifications, thus establishing its clear superiority over conventional treatments. However, every advantage comes with a corresponding drawback for the CRISPR-Cas9 technology. Its primary concerns revolve around the degree of control that scientists and researchers have over this technology, as well as its potential to give rise to the so-called 'designer babies'. These issues will be thoroughly explored in this paper.

Introduction

Epigenetics delves into the study of how behavior and environmental factors can modify genes. While genetic changes modify the DNA sequence itself, epigenetic changes alter how the body reads and interprets that sequence. So in contrast to genetic changes, epigenetic changes are not permanent and can be reversible under certain circumstances. These changes often begin before birth and persist throughout an individual's lifetime. Certain germs and bacteria can weaken the immune system by causing epigenetic changes, and specific mutations can increase the risk of developing cancer. Moreover, organisms can inherit hereditary traits and genetic disorders from their parents, and a pregnant woman's behavior and surrounding environment can impact her baby's epigenetics. Epigenetic changes encompass various processes, such as DNA methylation, histone modification, and the involvement of non-coding RNA. These mechanisms play essential roles in regulating gene activity and contribute significantly to an individual's overall growth, development and future health outcomes [1]. This paper will focus on how the environmental pollutant benzene possesses the potential to induce epigenetic alterations to the initiation and progression of AML. Additionally, this paper will compare the traditional treatment, CAR T-cell therapy, with a more novel and innovative therapeutic approach, CRISPR-Cas9.

A Background of Benzene-Induced Acute Myeloid Leukemia (AML)

This section will delve into the fundamental aspects of AML and explain how an environmental toxin such as benzene can trigger the disease. AML occurs through a combination of genetic and epigenetic alterations in patients, paving

the way for a variety of potential treatments, both current and new, which will be further discussed in subsequent sections of this research paper.

Benzene (C6H6) is a well-established chemical carcinogen [2]. It exists as a colorless or light yellow liquid with a pleasant odor at room temperature, but can also be characterized by its rapid evaporation in air, as well as its limited solubility and high buoyancy in water. Notably, benzene is highly flammable and can originate from both anthropogenic and non-anthropogenic sources. Natural sources such as volcanic activity and forest fires contribute to the presence of benzene in the environment. However, the primary source of benzene is through industrial processes. These include emissions from the combustion of coal and crude oil, activities related to benzene waste and storage, exhaust emissions from motor vehicles, evaporation from gasoline service stations, and even the combustion of cigarette smoke. Despite being a recognized carcinogen, benzene continues to be extensively utilized. In the United States, benzene ranks among the top 20 chemicals in terms of production volume [3]. Industries frequently employ benzene in the production of various chemicals, subsequently used for the manufacturing of plastics, resins, nylon, and synthetic fibers, as well as for the production of specific rubbers, lubricants, dyes, detergents, pharmaceuticals, and pesticides [3].

A more pressing issue arises when exposure to diverse environmental toxins becomes a catalyst for specific hematologic malignancies. These malignancies originate in blood-forming tissue such as the bone marrow and immune cells. The three main types of hematologic malignancies are leukemia, lymphoma, and multiple myeloma. Prolonged exposure to elevated levels of airborne benzene increases the risk of leukemia, a form of cancer that impacts red blood cells, white blood cells, and platelets. But more specifically, Acute Myeloid Leukemia (AML) has been found to be increasingly prevalent among people exposed to benzene. AML is the most prevalent type of acute leukemia among adults and is characterized by rapid disease progression if left untreated. AML specifically manifests when the bone marrow produces a surplus of abnormal blood cells. Several factors can increase the risk of developing AML, including smoking, previous chemotherapy treatments, and exposure to radiation. Common symptoms associated with AML include fever, fatigue, and easy bruising or bleeding. Diagnosis of AML relies on tests that examine the blood and bone marrow, while disease prognosis can be influenced by various factors [4].

The development of AML is a significant concern. Normally, the bone marrow undergoes a process where immature blood stem cells gradually transform into mature blood cells. During this transformation, a blood stem cell has the potential to become either a lymphoid stem cell, resulting in lymphoblasts that eventually mature into white blood cells, or a myeloid stem cell. Myeloid stem cells, in turn, can develop into red blood cells that are responsible for transporting oxygen and essential substances throughout the body, or platelets which play a crucial role in forming blood clots. Myeloid stem cells can also differentiate into myeloblasts and then further mature into granulocytes which aid the body's defense against infection and diseases [4].

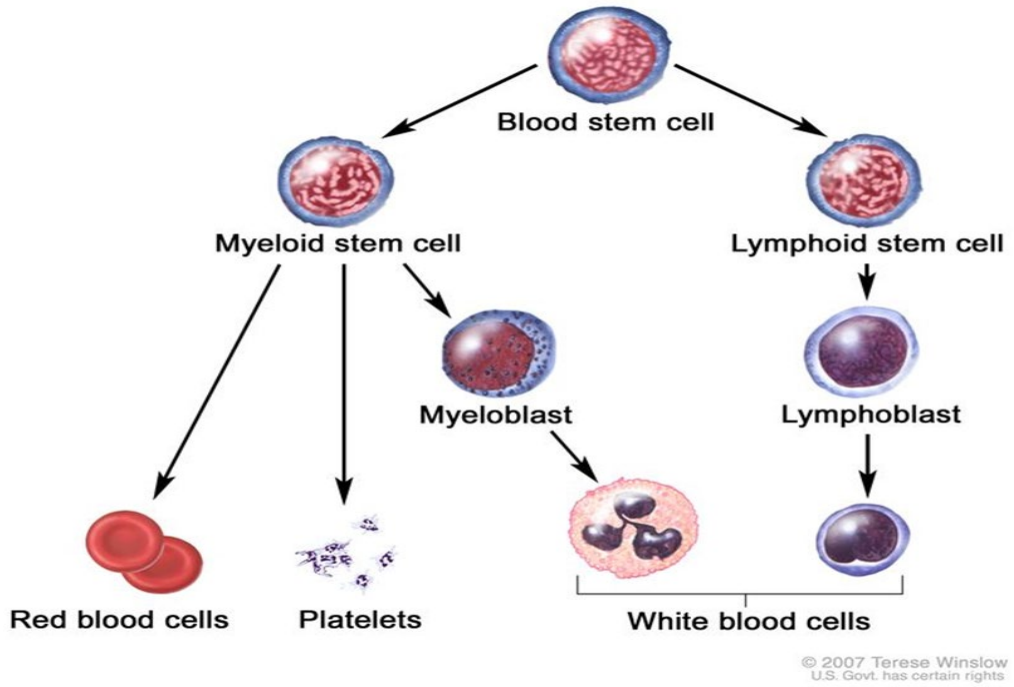

Figure 1. Blood cell development. This illustration depicts the transformation of a blood stem cell into a mature blood cell type. Adapted from National Cancer Institute. (2019, July 23). Adult Acute Myeloid Leukemia Treatment (PDQ®)–Patient Version. National Cancer Institute; Cancer.gov. https://www.cancer.gov/types/leukemia/patient/adult-aml-treatment-pdq

In the case of AML, myeloid stem cells fail to progress into healthy and mature white blood cells but instead remain as immature myeloblasts. AML can be triggered by an overabundance of stem cells transforming into abnormal red blood cells or platelets referred to as leukemia cells or blast cells. The accumulation of these leukemia cells in the bone marrow and bloodstream reduces the space available for healthy mature blood cells, leading to complications such as infection, anemia, or easy bleeding [4].

Similar to other cancers, AMLcan arise due to genomic mutations. Typically, these mutations activate oncogenes while simultaneously inactivating tumor suppressor genes which normally regulate cell division and trigger programmed cell death (apoptosis) when necessary. In AML cells, certain genes such as fms-like tyrosine kinase 3 (FLT3), tyrosine-protein kinase KIT (c-KIT), and rat sarcoma (RAS) often undergo alterations, leading to uncontrolled cell growth and disruption of the normal maturation process of bone marrow cells. Chromosomal changes such as translocations are also prevalent in AML cases. Translocations occur when a segment of a chromosome breaks off and attaches to a different chromosome, potentially affecting nearby genes. This can activate oncogenes or deactivate genes like runt-related transcription factor 1 (RUNX1) and retinoic acid receptor, alpha (RARa), which are essential for blood cell maturation. Furthermore, deletions may occur when a section of a chromosome is lost or removed, leading to the loss of a tumor suppressor gene that helps regulate cell growth. Inversions can also happen, wherein part of a chromosome gets reversed in order, resulting in the loss of one or more genes due to the cell's inability to interpret the instructions in reverse. Additionally, addition or duplication events can result in an extra chromosome or additional segments of a chromosome, leading to an excessive number of certain genes within the cell. This can be problematic if any of these genes happen to be oncogenes. Numerous genomic mutations or changes in chromosomes can contribute to the development of AML, with the specific patterns varying depending on the individual patient and the type of AML they have [5,6].

But more importantly, the environmental pollutant benzene also possesses the capacity to induce AML in patients through a variety of epigenetic modifications, without altering the DNA sequence itself. p16 (MTS 1) and p15 (MTS 2) function as tumor suppressor genes (TSG), exerting control over cell cycle progression at the G1 checkpoint. Positioned on chromosome 9p21, both p15 and p16 play crucial roles, with alterations evidenced across a spectrum of human malignancies and in various human cancer cell lines including AML. Benzene possesses the potential to adversely modify the expression of p15 and p16 genes by means of DNA methylation. In individuals with benzene poisoning, the presence of DNA methylation in the promoters of p15 and p16 genes correlates with an increased susceptibility to conditions like AML, myelodysplastic syndrome, and lymphoma [7].

Current Treatment of AML

The immune system plays a crucial role in the body's defense against many different disease-causing pathogens such as bacteria, viruses, parasites, and cancer cells while protecting healthy tissue. Certain immune cells possess a remarkable capability to identify cancer cells as abnormal and eliminate them through apoptosis promptly. However, this natural response alone is often insufficient to completely eradicate cancer. Therefore, certain treatments focus on harnessing the power of the immune system as a tool to combat cancer effectively [8].

A lymphocyte is a type of white blood cell found in the immune systems of most vertebrates. These cells play a crucial role in the body's defense against cancer, foreign viruses, and bacteria (antigens) by being able to remember every antigen they encounter. As a result, some lymphocytes transform into memory cells after an encounter. When memory cells encounter the same antigen again, they quickly recognize it and mount a rapid immune response. This phenomenon explains why certain infections, such as measles or chickenpox, are not experienced multiple times and how vaccination can prevent specific diseases.

Lymphocytes comprise B lymphocytes (B cells), T lymphocytes (T cells), and innate lymphoid cells (ILCs). B cells produce proteins called antibodies that target viruses, bacteria, and other foreign invaders through three primary mechanisms: 1) Antibodies are secreted into the bloodstream and mucosa, where they bind to and inactivate foreign substances such as pathogens and toxins; this process is known as neutralization, 2) Antibodies activate the complement system to destroy bacterial cells by creating openings in their cell walls; this process is known as lysis, and 3) Antibodies facilitate the engulfment of foreign substances by phagocytic cells, thus enhancing the process known as opsonization [9]. These B cells possess receptors on their surfaces where antigens can bind. In response to antigens, B cells can act in two ways. In the primary immune response, some B cells transform into memory cells while others become plasma cells that produce specific antibodies; the production of sufficient quantities of these antibodies may take several days. In the secondary immune response when encountering the same antigen again, memory cells rapidly multiply and differentiate into plasma cells, efficiently producing the appropriate antibody to combat the antigen [10,11].

On the other hand, T cells regulate the immune system's response and directly target infected cells such as tumor cells. They play a crucial role in killing infected cells and managing the body's immune response to foreign substances. To become activated, most T cells require assistance from another immune cell. Once activated, T cells multiply and differentiate into three main types: cytotoxic (killer) T cells, helper T cells, and regulatory (suppressor) T cells. Cytotoxic T cells attach to antigens on infected or abnormal cells, leading to the death of these cells by creating holes in their membranes and inserting specific enzymes. Helper T cells assist other immune cells, aiding B cells in producing antibodies against foreign invaders and activating cytotoxic T cells. And lastly, regulatory T cells produce substances that help terminate the immune system's response to an attack and sometimes prevent harmful responses from occurring [10,11].

Chimeric antigen receptor T-cell therapy (CAR T-cell therapy) is an innovative form of immunotherapy designed to combat cancer using modified T cells. This therapy involves engineering T cells to express chimeric antigen receptors (CARs) proteins that are able to target a specific antigen on cancer cells. The process of CAR T-cell therapy is categorized as adoptive cell transfer [12]. The procedure generally begins with the extraction of T cells

from the patient through leukapheresis, a process where blood is drawn from the patient and T cells are isolated from the other blood components. In the laboratory, the T cells are then genetically altered and modified to express chimeric antigen receptors (CARs) capable of recognizing specific proteins, known as antigens, on the surface of cancer cells. Subsequently, the modified CAR T-cells are cultured and allowed to proliferate, generating a larger population of CAR-expressing T cells. Before the CAR T-cells are reintroduced into the patient, they may also undergo a conditioning regimen, which could involve chemotherapy or radiation therapy. This conditioning helps create a more favorable environment for the CAR T-cells to function optimally. Once the CAR T-cells have been multiplied, and the patient is prepared, the engineered cells are infused back into the patient's bloodstream. Once inside the body, the CAR T-cells are able to effectively target and attach themselves to cancer cells expressing the specific antigen, leading to the destruction of these cancerous cells [12]. Numerous studies have proven that 9 out of 10 individuals with Acute Lymphoblastic Leukemia (ALL), a condition closely related to AML, achieved full remission through CAR T-cell therapy, even if their cancer was unresponsive to prior treatments or had relapsed [13]. As a result, CAR T-cell therapy has exhibited impressive effectiveness and success rates in addressing specific blood cancers, including AML. This has offered newfound hope and optimism for patients who have not responded to conventional treatments or have suffered relapses following previous therapies.

Challenges

Although CAR T-cell therapy has achieved numerous successes, the treatment also has significant side effects, some of which can be severe or life-threatening. The primary causes behind these side effects stem from an exaggerated and overactive immune response known as cytokine release syndrome (CRS). This reaction occurs when the immune system reacts more aggressively than necessary to certain infections or immunotherapy drugs and medications, leading to a widespread and systemic inflammatory response throughout the human body [14]. Additionally, neurologic toxicities, or neurotoxicity, may arise due to exposure to natural or synthetic (man-made) toxic substances, known as neurotoxicants, causing a disruption in the normal functioning of the nervous system. Consequently, this disruption can result in damage to, or even the demise of, nerve cells, or neurons, crucial for transmitting and processing signals within the brain and other components of the nervous system [15,16]. As a result, close monitoring and proper management are vital both during and after undergoing CAR T-cell therapy [17].

The field of CAR T-cell therapy is constantly evolving, with ongoing research aimed at expanding its applications to other types of cancer while enhancing its safety and efficacy. However, the therapy's severe side effects still leave a need for a safe and effective AML treatment option.

New Treatment: CRISPR-Cas9

Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 (CRISPR-Cas9) is a powerful genome editing technology used to selectively modify the DNA of living organisms. The system is based on a natural defense mechanism used by bacteria to protect themselves from viral infections. CRISPR constitutes a group of DNA sequences present in the genomes of prokaryotic organisms. These sequences contain short repetitive base sequences and play a crucial role in the defense mechanisms against viruses in prokaryotes. At the core of CRISPR-Cas9 is the Cas9 enzyme, a 160-kilodalton protein that is able to cut DNA and modify the genome of a cell. To achieve gene editing, CRISPR-Cas9 precisely cuts DNA and then harnesses the cell's natural DNA repair process. [18-21].

CRISPR-Cas9 initiates its process by identifying the target DNA sequence. Scientists first design a small piece of RNA called a guide RNA (gRNA) that is complementary to the specific sequence they wish to modify in the target DNA. This target sequence can be from any organism and can target any gene of interest. Acting as molecular scissors, the Cas9 protein binds to the gRNA and navigates to the target DNA sequence. The Cas9-gRNA complex functions like a GPS, homing in on the DNA region that matches the gRNA. Upon locating the target DNA, the Cas9

protein cleaves both strands of the DNA double helix at a precise location, resulting in a DNA break. Subsequently, the cell's natural repair mechanisms come into play. There are two main repair pathways: Non-Homologous End Joining (NHEJ) and Homology-Directed Repair (HDR). In the Non-Homologous End Joining (NHEJ) pathway, small insertions or deletions (indels) are introduced at the break site, often leading to frameshift mutations that can disrupt the gene's function. In the Homology-Directed Repair (HDR) pathway, a template, usually a synthetic DNA fragment provided by researchers, guides the repair process. This allows scientists to introduce precise modifications to the target DNA by providing a template with the desired genetic changes. By harnessing the DNA repair mechanisms, researchers can either disrupt gene function by inducing mutations or replace specific DNA sections with new sequences. This capability enables them to effectively "edit" the genetic information in the targeted region [18-21].

The CRISPR-Cas9 technology enables three primary categories of genetic edits: disruption, deletion, and correction or insertion. In the disruption process, a single cut is made, leading to non-homologous end joining, which can result in the addition or removal of base pairs. Consequently, the original DNA sequence is disrupted, causing gene inactivation. During the deletion process, a larger DNA fragment can be eliminated by employing two guide RNAs that target separate sites. After cleavage at each site, non-homologous end joining brings together the separate ends, resulting in the deletion of the intervening sequence. Lastly, in the correction or insertion process, the introduction of a DNA template alongside the CRISPR-Cas9 machinery allows the cell to rectify a gene or even introduce a new gene through a mechanism known as homology-directed repair [18-21].

Benefits and Drawbacks of CRISPR-Cas9

Despite being awarded the 2020 Nobel Prize in Chemistry [22], the CRISPR-Cas9 technology continues to spark numerous discussions due to its multitude of advantages and disadvantages. CRISPR is known for its precision and versatility as it enables targeted modifications in various organisms. These abilities hold the potential for medical advancements and research. However, concerns about unintended genetic changes, ethical dilemmas, and regulatory challenges highlight the need for cautious and responsible use.

The CRISPR-Cas9 system has an extensive range of applications across various industries, including cancer therapeutics, genetic disease cures, drug research, and pest-resistant crops. By utilizing CRISPR, new immunotherapies can be developed to treat cancers like AML, where genetically modified T cells, similar to CAR T-cell therapy, are employed to target and eliminate cancer cells. CRISPR can also be employed to target genes responsible for genetic disorders such as diabetes and cystic fibrosis. Additionally, many modern scientists anticipate that CRISPR could accelerate the drug discovery process due to its affordability, precision, and ease of use, with some pharmaceutical companies already integrating it into their research and development phases. Furthermore, according to CRISPR pioneer Jennifer Doudna, CRISPR's genome editing capabilities could address many agricultural challenges, particularly concerning pests and nutrition, especially in light of climate change and population growth. Some crops edited with CRISPR have even been exempted from GMO regulation due to the technology's accuracy [23,24].

However, CRISPR-Cas9 also comes with several drawbacks. One significant concern involves the potential manipulation of germ-line cells, which raises the prospect of creating "designer babies" carrying desired traits passed on to subsequent generations. This process, known as germline editing, entails making specific genetic modifications to human embryos and reproductive cells. While some countries have implemented outright bans on germline editing, others lack clear guidelines, leading to varying approaches, such as considering exemptions for certain hereditary disorders in the United States and the United Kingdom [25,26]. Another major issue is the potential misuse of CRISPR as a bioweapon. Given its relative affordability and simplicity compared to other genetic engineering tools, CRISPR could be appealing to terrorist organizations seeking to genetically modify bacteria or viruses for biological attacks on human populations. In response to this concern, the United State’s Defense Advanced Research Projects Agency (DARPA) has invested significant resources to research and explore ways of enhancing the safety and precision of gene editing technologies like CRISPR. The three main objectives of DARPA's efforts are: to develop processes that

allow for greater control of genome editing in living systems, to create countermeasures that safeguard genome integrity in populations, and to investigate methods for removing engineered genes from living systems to mitigate potential consequences [23,24].

Conclusion

In conclusion, this research paper clearly demonstrates how benzene possesses the potential to induce epigenetic modifications that contribute significantly to the development of AML cancer. Prolonged exposure to this ubiquitous environmental pollutant can seamlessly translate into the dysregulation of key cellular processes, ultimately leading to the malign transformation of hematopoietic cells—facilitated exclusively through the mechanism of epigenetics, particularly DNA methylation.

Nonetheless, the revolutionary gene-editing tool known as CRISPR-Cas9 has allowed us to effectively target and modify specific DNA sequences in a precise and programmable manner. This innovation presents a new yet promising avenue for cancer treatment, as it has enabled us the ability to disrupt or modify genes pivotal to cancer's genesis and progression. These genes encompass those governing cell proliferation, DNA repair, and the suppression of tumors.

Furthermore, environmental activism offers an additional solution that directly addresses the heart of this issue. By implementing rigorous environmental regulations on specific industrial processes, we can limit the release of benzene into our atmosphere in the first place, effectively minimizing the risk of developing AML cancer through this environmental pollutant.

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