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Gene Therapy for Cancer Treatment

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Abstract

Cancer is a genetic disease in which cells grow uncontrollably and can spread throughout the body. Cancer cells have distinguishing traits which facilitate their unlimited growth; these traits can be used in the development of gene therapy cancer treatment, which includes: CRISPR, a gene-editing tool that can precisely modify human DNA; Kymriah, which induces T cells to kill cancer cells; Gendicine and zinc metallochaperones, which utilize p53, a protein vital for the destruction of cancer cells. Scientists continue to improve gene therapy treatments, making them available to more patients and decreasing the toxicity of treatments.

Introduction

Cancer is a genetic disease in which cells grow uncontrollably and can spread throughout the body. Human cells grow and multiply through a process called cell division, in which new cells take the place of old cells, and, normally, the body rids itself of cells that have damaged DNA. When genes, which control cell division, are damaged, abnormal or damaged cells may develop. Errors that occur as cells divide, damage to DNA caused by toxic substances such as tobacco smoke and UV rays, and inherited mutations are genetic changes that can cause cancer. Damaged cells can form lumps of tissue and tumors, which can be benign or cancerous.

Cancer cells have distinguishing traits which facilitate their unlimited growth. Malignant tumors invade healthy tissue and can spread to other areas of the body, metastasize and form additional tumors; metastatic cancer is classified according to its origin. Cancer cells use a variety of tactics that differentiate them from normal cells and allow them to take control; they ignore signals telling them to stop growing or die by apoptosis; they communicate with blood vessels to grow toward tumors to help them survive; they hide from the immune system to prevent their destruction and can trick the immune system into protecting tumors; they can also change their chromosomes and rely on different nutrients. In the development and treatment of cancer, the differences between cancer cells and normal cells are used to target the abnormal cells. (National Cancer Institute, 2021) For best results, a variety of cancer treatments are used in combination; however, many treatments currently available have debilitating side effects. Gene therapy, an experimental treatment for various diseases, specifically focuses on cancer and is currently undergoing clinical trials in the United States. Scientists believe that, with additional research and advances, gene therapy will replace more invasive cancer treatments.

Methods

Data was compiled using the National Library of Medicine, PubMed databases, and Science.org.

Discussion

CRISPR

A gene-editing tool, CRISPR, which can precisely modify human DNA, was developed. CRISPR, inspired by nature, imitates the mechanisms of microbes. In nature, microbes capture small segments of intruder DNA and store them. If the same invader attacks again, those segments of DNA help an enzyme, Cas, locate and destroy the invader's DNA. CRISPR, using a cutting enzyme, Cas 9, uses virus RNA as a guide which mirrors the DNA of the gene being edited. When the RNA matches with the target gene, Cas 9 cuts the DNA. Scientists have been testing CRISPR's effect on editing immune cells to improve recognition and then attack cancer cells, and they have also used CRISPR to detect specific targets, such as DNA, from cancer-causing viruses and RNA from cancer cells. Researchers can use hundreds of guide RNAs to edit and evaluate hundreds of genes at a time and can pick out genes that will be effective drug targets. CRISPR is a valuable tool for future cancer treatment.

While solutions are being developed, technical limitations, such as off-target editing, negative immune response to the gene-carrying virus, and fear of alterations in the DNA of the germ cells have slowed CRISPRs entrance into the market. At times, CRISPR cuts untargeted DNA and off-target editing, and scientists are worried that off-target edits could turn cells cancerous. Therefore, to improve the accuracy of Cas 9 and the guide RNA, researchers use a virus that can only infect one organ; while this makes CRISPR safer, it also limits the number of viruses that can be used. Nanocapsules have also been used to deliver CRISPR components to specific cells. Another concern with gene editing is that CRISPR may accidentally edit sperm or egg cells, and the changes will become hereditary. Therefore, to prevent changes to germ cells, the cells are edited outside the body only. Additionally, the immune system can attack gene-carrying viruses. In 2001, a patient died when his immune system attacked a gene-carrying virus, and scientists fear that CRISPR edited viruses may be attacked. However, scientists have developed a better understanding of viruses and choose those that appear to be safer, and they continue running clinical trials to test this improved knowledge.

The first CRISPR trial in the United States to test the safety and efficacy of CRISPR edited T cells that help destroy cancer was performed in 2019. The collection of preclinical data began in 2016, and it was over two years before the federal government approved phase 1 of the trial. (Penn Medicine News, 2020) Scientists performed four edits on the T cells: the first two helped the T cell survive; the third reduced toxicity; and the fourth enhanced

the cell's tracking mechanism by adding a gene to locate tumors. Usually, edited T cells survive for approximately a week, but the first two modifications allowed the cells to last for at least nine months. A lentivirus was used to insert the tracking mechanism, which informs the edited T cells to target the NY-ESO-1 antigen. Patients had to have a molecule called HLA-A*02:01 to be approved for the trial; this molecule activates the CRISPR edited T cells, which, unlike CAR T cells, are not active on their own. Two patients with refractory, advanced myeloma and one with metastatic sarcoma met the trial requirements. Results indicated that the treatment was safe, and side effects were likely due to prior doses of chemotherapy. No evidence of an immune reaction to the CRISPR-edited cells was reported. However, only ten percent of the T cells used for the therapy had the four intended genetic edits. Some off-target modifications were found, none of which appeared to have turned cancerous. While no adverse reactions were evident, the CRISPR treatment had little effect on the tumors. The tumors of two of the patients in the trial stopped growing for a short period of time but then resumed growth. The tumors of the third patient were not affected at all. (Stadtmauerer et al., 2020). The long-term effects of this therapy still need to be monitored. To fully assess the efficacy of CRISPR therapy experience with more patients, using advanced editing techniques and monitoring the results for a longer period of time was needed. In March of 2022, using CRISPR- based technologies, Intellia Therapeutics, Inc., a clinical-stage genome editing company, announced that T cell therapy, NTLA-5001, designed to target Wilms' Tumor, WT1, antigen, found in acute myeloid leukemia, was administered to the first patient. The therapy was developed using Intellia's advanced lipid nanoparticle cell engineering platform, which was designed to improve edited cell performance (Intellia Therapeutics, Inc., 2022).

Acute myeloid leukemia and some other forms of cancer are distinguishable by the increased level of Wilms' Tumor 1 antigen, which encodes a transcription factor that displays an important part in cell growth and differentiation. (Haruo, 2010). NTLA-5001, designed for acute myeloid patients with HLA-A*02:01 allele, promotes T cell survival and accurately targets intracellular tumor antigens. To improve T cell receptor-based therapy, T cell receptors (TCRs) specific for shared oncogenic antigens are still needed, and the redirection of T cell specificity, while promoting T cell survival, requires modification. Using fifteen healthy donors, nineteen specific TCRs for Wilms' tumor antigen one were isolated. The TCRs recognized various peptides which restricted common human leukocyte antigen alleles and exhibited a wide range of effective avidities. The researchers then selected five high-avidity HLA-A*02:01- restricted TCRs, and primary acute myeloid leukemia blasts processed the TCRs. Using CRISPR-Cas9 gene-editing tools, the researchers combined TCR-targeted integration into the TCR alpha constant locus with TCR beta constant knockout to prevent TCR alpha beta mispairing and to increase the TCR activity and expression. The engineered lymphocytes were placed into memory stem T cells. A distinct WT137-45 specific TCR exhibited antigen-specific responses and successfully destroyed acute lymphoblastic leukemia blasts and glioblastoma cells in vitro and in vivo. No off-tumor toxicity was present. Researchers are now engineering T cells to express the effective receptor, which is being clinically developed for acute myeloid leukemia immunotherapy and shows potential in the treatment of other WT1 expressing tumors (Ruggiero et al., 2022).

The FDA approved Phase 1/2a study to assess the safety and efficacy of a single dose of NTLA-5001 in adults with acute myeloid leukemia. First, patients will receive standard cancer therapy and then a single dose of NTLA-5001. If proven safe, the researchers will then administer additional doses of NTLA-5001 to a group of patients with a mild number of tumors and one group with a larger amount. Another phase will expand to include up to fifty-four patients. After the first two phases are complete and the dosages are determined for each group, the trial will expand to include more patients and further assess the safety of the treatment. Based on the results of the preclinical trial, Intellia believes the therapy will lead to a safer, more efficacious cancer treatment. (Intellia Therapeutics, Inc., 2022)

Kymriah

Kymriah, a cell-based gene therapy for patients up to age twenty-five with B cell acute lymphoblastic leukemia, is used for refractory, in relapse after transplant, or in second or later relapse, and for adult patients with relapsed or refractory diffuse large B-cell lymphoma after two or more lines of systemic therapy. The active substance in Kymriah is tisagenlecleucel; the drug involves reprogramming the patient's T cells to identify and eradicate CD19 expressing cells, which is done by adding a replication-defective, self-inactivating lentivirus vector containing an anti-CD19 CAR transgene. When CAR binds with CD19-expressing cells, the transgene transmits a signal to induce T cell expansion, activation, and target cell elimination. Monocytes, natural killer cells, and B cells may also be added to the drug. The number of T cells added is patient-specific. Based on its high success rate in driving the cancers into complete remission with minimal

residual disease, on August 30, 2017, tisagenlecleucel became the first gene-modified cell therapy to gain FDA approval. However, the therapy is not without side effects. The most frequently reported reaction to the drug was cytokine release syndrome (Clinical Cancer Research, 2019). Cytokine release syndrome is caused by a large, rapid release of cytokines into the blood from immune cells affected by the immunotherapy" and is characterized by fever and organ failure (National Cancer Institute). The cytokine release syndrome that patients experienced was reversible in most cases and managed with supportive care and anti-cytokine therapy. Half of those who suffered from cytokine release syndrome were admitted to an intensive care unit and, on average, remained there for one week. Two patients, out of the 63 in the trial, died within 30 days of infusion. One patient died with cytokine release syndrome and progressive leukemia, and the second with resolving cytokine release syndrome, abdominal compartment syndrome, coagulopathy, renal failure, and intracranial hemorrhage. Neurological side effects were also present, a majority of which were resolved (The Oncologist, 2020). Since approval, the treatment has not been expanded to a wider age range since its safety has not been established (Clinical Cancer Research, 2019). The use of the treatment is limited because potential benefits do not always outweigh the risks.

Research on the long-term efficacy of Kymriah recently highlighted two patients who continue to display a CAR T cell response ten years post-treatment. Both patients had been diagnosed with chronic lymphocytic leukemia, and when their cancers no longer responded to standard therapy, they became the first participants in the clinical trial of Kymriah. The patients went into remission that year, and in a recent analysis, researchers observed that the modified CAR T cells had a highly activated CD4+ T cell population, which have become dominant in both patients. The data implies two phases of the response to CAR T-cell therapy: one phase is dominated by killer T cells, and the long-term phase is dominated by CD4+ T cells. The CD4+ T cells, which became the majority of T cells and increased their dominance with time, continued to display mechanisms that destroy tumors and continued growth, which demonstrates the efficacy of CAR T cells. This CD4+ T cell dominance led researchers to believe that CD4+ T cells may be foremost in distinguishing T-helper from T cytotoxic cells. (Penn Medicine News, 2022).

P53

Research has shown that mutant P53 is a leading cause of cancer. Professor David Lane discovered P53 and dubbed it the "guardian of the genome" as it prevents mutations from passing down to daughter cells. The wild-type p53 protein is activated by cellular stress, such as hypoxia, DNA damage, and oncogenic stress, and mediates cell-cycle arrest and DNA repair or induces apoptosis depending on the degree of cellular stress (Zhu et al., 2020). The Cancer Genome Atlas program analyzed Tp53 mutations in 10,225 patients with 32 different forms of cancer to study the effects of the mutation (Kandoth et al., 2013). The mutation was present in 3,786 patients, and the mutation frequency varied with cancer type, ovarian and uterine cancer showing ninety percent incidence. In contrast, other cancer types had less than five percent incidence. Analysis of the Tp53 mutation found that it causes instability of chromosomes, which included increased oncogenes and deletion of tumor suppressor genes (Donehower et al., 2019). Mutant Tp53 was found in over half of human cancers, leaving the body unprotected against tumors.

Another study supported the efficacy of p53 restoration. They injected drugs that restore mutant p53 in mice with lymphomas and sarcomas. The results indicate that the restored mutant p53 led to shrinkage of lymphomas and sarcomas without damaging healthy cells (Ventura et al., 2020). The data also implies that drugs that restore mutant p53 in humans can shrink tumors as well.

Mutations in Tp53 cause the mutant gene to survive and take over. Mutant p53 acquires gain of function activities which leads to its dominance. Mutant p53 can interact with many transcription factors, which alters transcription, cell cycle, apoptosis, and cancer cells metabolism. Mutated p53 genes differ from most mutations because they produce a single amino acid substitution in the mutant protein. In addition, mutant p53 alters the cellular metabolism of glucose, lipid, and nucleotides, which correlates with the Warburg effect, that rapidly dividing tumor cells rely mainly on glycolysis to meet their high energy demand. The changes can lead to metastasis and chemotherapy and radiation resistance. Researchers found that mutant p53 ignores anti-growth signals and is responsible for the unlimited replication of tumor cells. Epithelial to mesenchymal transition is a critical factor in metastasis, allowing cells to gain the ability to migrate and invade. Chemotherapy and radiation are used to treat metastatic cancer; however, since mutant p53 activates MDR1, a gene that helps resist these therapies is activated by p53. Restoration of the wild-type p53 could end the activity of MDR1 by reducing its phosphorylation and thus increase the efficacy of chemotherapy and radiation. Professor David Lane predicted that many more drugs to target p53 would be developed in the future (Lane, 2010).

Gendicine

Gendicine, a gene therapy that utilizes p53 to treat head and neck cancer, was developed by Shenzhen Sibiono GeneTech Co. Ltd. and approved by the China Food and Drug Administration (CFDA) in 2003. Gendicine is an adenovirus vector, most commonly used for gene therapy due to its high gene-transfer efficiency, large gene-carrying capacity, selective gene delivery, mild cytotoxicity, potential therapeutic immunogenicity, ease of construction and manipulation, and cost-effective manufacture, that delivers wild type human p53 to tumor cells. Ad-p53 can boost the immune system to fight cancer cells by activating cytokine genes, tumor antigen genes, and co-stimulatory molecule genes. While other Ad-p53 drugs have undergone preclinical and clinical trials, Gendicine is the only one to have obtained approval but only in China.

The CFDA approved Gendicine because it increases the efficacy of chemotherapy and radiation when used in combination, and, since then, it has also been proven successful in the treatment of other cancers. The delivery of Gendicine is minimally invasive and injected intratumorally, intracavity, or intravascularly. Thirteen published studies have shown that standard therapies combined with Gendicine yield significantly longer survival rates. Gendicine, as a primary treatment, can also be used for ovarian cancer, malignant pleural effusions, or peritoneal ascites. The major adverse effect of Gendicine is fever within 24 hours of administration, which occurred in fifty to sixty percent of patients but was easily resolved. It is thought that the feve that many patients develop after treatment may have a positive effect; it alludes to the possibility that rAd-p53 can induce an immune response that kills tumor cells (Bioxue et al., 2016). Immune responses to adenoviral vectors have been studied extensively. Most patients have been exposed to adenovirus and have antibodies to neutralize the virus; nonetheless, the clinical efficacy of Ad5-based therapies appears effective and does not elicit an adverse immune response. In clinical trials, the anti-tumor effect of Gendicine was not at all inhibited by pre-existing antibodies. The anti-vector neutralizing antibody levels increased in patients after receiving Gendicine with no negative outcome. Over the course of twelve years, thirty Chinese clinical studies have been published, and approximately 30,000 patients have been given Gendicine, which provides strong evidence of its safety. Nonetheless, researchers continue to work on improving the safety and efficacy of Ad-vectors (Zhang et al.,2018).

Domestically, the FDA is more cautious than the CFDA when approving new drugs. Ad-p53, Gendicine in China, was originally developed by Introgen Therapeutics and Gendux under the name Advexin in the United States (Zhang et al., 2018). Advexin was developed for the treatment of head and neck cancer and Li-Fraumeni syndrome, a rare autosomal dominant mutation that increases the risk of developing cancer (Chin-Hang Kong, 2009). The Phase III trial conducted by Introgen compared the efficacy of Advexin with methotrexate, a chemotherapy drug. Patients, with p53 profiles that were positive for Advexin efficacy, had increased survival rates. Patients, with p53 profiles negative for Advexin efficacy, had increased survival following treatment with methotrexate. Outcomes showed that both Advexin and methotrexate increased survival depending upon p53 positive and negative profiles. Biomarker analysis indicates that Advexin suppresses tumors in negative p53 profiles, but the FDA refused to approve Advexin, despite having accepted the data of the biomarker. However, the refusal may be due to Advexin's inability to improve survival compared to standard therapy. The FDA then suspended the trial for Advexin in the United States since Introgen's Biologics License Application for the therapy was incomplete. The FDA has authorized the use of Advexin on a compassionate basis for patients with Li- Fraumeni syndrome under authorized protocol. Introgen intends to appeal the decision against Advexin (Chin-Hang Kong, 2009).

Shortly after launching Gendicine, the Chinese State Food and Drug Administration (SFDA) approved type 5 Ad derivative of E1B-55 kDa molecule for head and neck cancer treatment which was also originally developed in the United States under the name ONYX-015. The Chinese obtained exclusive license of ONYX-015 in the world and labeled it Oncorine. The United States suspended a phase III study of ONYX-015 to treat head and neck cancer. China's trials of Oncorine to treat head and neck cancer had similar results to that of ONYX-015 in the United States. Ad vectors are injected intratumorally and are well tolerated by patients; however, efficacy for some advanced cancers would increase if treatment were delivered intravenously. On target specificity of tumors is an area that still needs additional investigation. Modification of the viral coat proteins will reduce toxicity and improve on-target specificity Trials using Ad-p53 in combination with standard therapy, done in both the United States and China, show similar results that the Ad-p53 or Ad derivative of E1b-55 kDa are safe and can be beneficial for patients who have not responded to standard therapies (Ma et al., 2009).

Despite the many trials that have been conducted in the United States and other Western countries, Ad-p53 products have not received government approval and cannot be marketed. One reason for the delay is the inadequate information from Chinese studies. The Chinese

reports only summarize case reports and do not include the long-term effects of the therapy or an adequate control group. The Chinese obtained very different outcomes in their trials with Ad vectors than trials done in Western countries. The significantly better outcomes seen in the Chinese studies may be due to the fact that they included early-stage patients, who should have been treated with standard therapies and instead treated with a combination of Ad-p53 and standard therapies. Unfortunately, additional errors were found in the Chinese trials (Bioxue et al., 2016). None of the studies that the researchers analyzed focused on the P53 mutation in patients with Malignant Pleural effusion, so the effect of the P53 mutation on the response to Gendicine treatment was not assessed. However, previous studies have shown that Ad-p53 can inhibit the growth of human lung adenocarcinoma cell line containing the mutant and wild-type p53 genes. Other drawbacks of the data are the small sample size, the studies were performed in China, and different responses to treatment may occur in patients from other countries (Bioxue et al., 2016). Outcomes of the United States and other Western studies should not be compared with the studies done in China.

Much of the clinical data in China has not been well reported at international conferences, and, until recently, researchers from other countries have been unaware of cancer-related gene therapy progress in China (Ma et al., 2008). However, cancer patients from all over the world travel to China to receive treatment. The results are reported in Chinese domestic journals, but access to the information is unavailable to non-Chinese medical scientists. On the other hand, the available clinical data from Chinese studies provide helpful leads for future clinical studies. The growing number of patients who receive gene therapy treatment in China increases the need for phase IV studies in the United States. If Chinese studies meet international standards, they will be able to contribute to gene therapy research. However, the director of China's State Food and Drug Administration was found guilty of bribery to approve new medicines, which has led to further concern in international medical and pharmacological societies. China's gene therapy companies claim they were not involved in the fraudulent activity; however, the gene therapy approval process in China must be better monitored. (Ma et al., 2008). Despite the drawbacks, the evidence present suggests that Gendicine is an effective and safe treatment that improves the quality of life compared with standard therapies alone (Bioxue et al., 2016).

Zinc Metallochaperones

Another p53-based gene therapy, zinc metallochaperones, has impacted the ability to repair mutant p53. Zinc metallochaperones regenerate the p53 mutation that occurs in the DNA-binding domain, thus allowing the p53 gene to transcribe RNA, preventing a loss of function by impairing the binding of zinc to the p53 protein. For proper folding, the p53 protein requires binding to a single zinc ion. Initially, scientists attempted to bind or modify the mutated p53 gene to make it function in the cell's environment; however, no functional compounds were found. A class of drugs was discovered, zinc metallochaperones, which changes the environment to accommodate the mutated protein rather than the protein itself (Blanden, et al., 2015).. Zinc metallochaperones reactivate p53 by restoring the wild-type structure by reestablishing zinc-binding, which changes the conformation of wild-type p53. The zinc metallochaperones also reactivate p53 through post-translational modifications brought on by cellular reactive oxygen species-ROS, which causes apoptosis of the cancer cell via p53 They bind zinc and other divalent metal ions, which are strong enough to remove serum albumin but weak enough to donate zinc to mutant p53. Further research led scientists to discover that the homeostatic mechanisms to maintain intracellular zinc levels are induced by zinc metallochaperones. Therefore, when zinc levels are stable, the homeostatic mechanisms can deactivate the zinc metallochaperones (Kogan & Carpizo, 2018). Research of the pharmacodynamics of zinc metallochaperones has led scientists to recognize that the ON/OFF switch mechanism of zinc metallochaperones allows for the brief reactivation of the p53 mutant and enables on-target efficacy, and avoids off-target toxicities. The alternate route, targeting the environment rather than the protein, has created a new method of drug development.

For the treatment of tumorigenic p53 mutations, researchers analyzed 20 of the most common mutations and found that eighty percent impair zinc affinity, thermodynamic stability, or both. Blandon et al. explain that for treatment, mutations will be classified into three groups that will be useful when categorizing patients. Synthetic zinc metallochaperones repair both mutations that decrease zinc affinity and mutations that destabilize DNA binding domains without impairing zinc binding. Zinc metallochaperones can repair mutations that are associated with new cancer cases in over 120,500 patients each year in the United States. (Blanden et al., 2020)

Preclinical studies of cancer models show that a zinc metallochaperone therapy, ZMC1, improved survival and inhibited tumor growth, specifically for the zinc-deficient allele. In the BRCA1-deficient breast cancer model, scientists discovered that ZMC1 and the PARP inhibitor Olaparib, an enzyme that helps repair DNA damage in

cells, combine to increase efficacy. Olaparib has been approved for the treatment of advanced BRCA1/2 mutant ovarian and breast cancers. Some tumors have developed resistance to Olaparib but are still affected by ZMC1 treatment. Researchers are testing combinations of ZMC1 and other chemotherapy drugs. (American Association for Cancer Research, 2020)

Scientists thought ZMC1 would work well with chemotherapy and radiation; however, their hypothesis was proven incorrect. The reason for the lack of cooperation was found in the reactive oxygen species activity, ROS, which negates the signal on p53 that is generated with chemotherapy and radiation. The signaling events that chemotherapy and radiation would normally induce to activate p53 are already being stimulated by ZMC1. ROS enables ZMC1 to act on its own but inhibits it from working together with chemotherapy and radiation. Although ZMC1 does not operate together with chemotherapy and radiation, it does work well with other targeted agents, and other zinc-binding agents work well with chemotherapy and radiation by inducing p53 signals on mutant p53. (Zaman et al.,2019).

Conclusion

Research has shown that gene therapy, including CRISPR, Kymriah, Gendicine, and zinc metallochaperones, can be effective in treating cancer. While scientists have developed an array of genetic treatments to attack cancer cells, many gene therapy treatments have not been brought to market for fear that the long-term effects could interfere with the human genome. Hopefully, with additional data and safety improvements, all patients will be able to benefit from gene therapy treatments in the near future.

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