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Emerging Role of Gene Therapy in Cancer

Jiahang Han

Department of Biomedical Engineering, The Pennsylvania State University, State College, US
jph6723@psu.edu

Abstract:

Cancer is one of the leading causes of death in the world, and researchers have been persistently studying the treatment of cancer. Although traditional treatment methods such as surgery, chemotherapy and radiotherapy are effective, they are often accompanied by serious side effects, drug resistance or limited effects on certain types of cancer. Therefore, it is urgent to develop more accurate and effective treatment plans. Cancer gene therapy is an innovative method to treat or prevent cancer by introducing or editing genetic material. Gene therapy brings new hope to the treatment of cancer, repairing mutant genes, activating the immune system or directly inducing cancer cell death. This review introduces some hot strategies of cancer gene therapy, including suicide gene therapy, tumor suppressor gene activation, immunotherapy, inhibition of oncogene activation, antiangiogenic gene therapy. The principles, application examples and advantages and disadvantages of these therapies in clinical research are also discussed. In addition, the challenges faced by gene therapy in improving safety and reducing side effects, and points out the direction of future research are also described, aiming to promote the transformation of these innovative therapies into conventional clinical applications.

Keywords: Gene therapy; treatment strategy; targeted delivery; clinical research.

1. Introduction

Cancer is a disease with a high mortality rate and difficult to treat in the world. Its morbidity and mortality rate are growing rapidly, causes about one in six deaths worldwide, posing a great threat to human health and life. Cancer, also known as malignant tumor, is caused by abnormal cell growth and is invasive and metastatic. The main classifications include cancer, sarcoma and cancerous sarcoma. The cause of cancer is usually due to the long-term co-action of chemical, physical, viral and other factors, causing a series of abnormal genetic changes.

The traditional methods of treating cancer mainly include surgery, radiotherapy and chemotherapy, which can be treated alone or in combination to achieve better treatment results. Radiotherapy refers to the elimination and eradication of primary or metastatic lesions of local tumors by radiation; Chemotherapy kills tumor cells with chemical drugs to effectively control tumor growth or kill tumors. For early cancer patients, surgical removal of the tumor or assisted by radiotherapy or chemotherapy, the cure rate is still very high. However, for patients with intermediate and advanced cancer, the morbidity and mortality rate are greatly improved. At the same time, traditional treatment methods also have obvious drawbacks: for example, radiotherapy and chemotherapy are fundamentally indiscriminate attacks on cells, which can damage healthy cells, which is a very obvious and very serious side effect. Second, drug resistance is also a great limitation of chemotherapy treatment, which also greatly reduces the treatment effect [1].

As a global health problem, human research on cancer treatment has never stopped. Gene therapy is one of the key research areas of researchers. Gene therapy treats diseases by operating specific sequences of DNA and directly adjusting the genetic structure. Genes are composed of coding regions, introns, promoters and terminators, which control protein synthesis and gene expression. Gene therapy uses these structures to restore the normal function of genes by introducing healthy genes into cells to replace defective genes, or using gene editing tools (such as CRISPR-Cas9) to repair mutations in DNA. In addition, gene therapy can inhibit the expression of harmful genes by interfering with RNA, or modify the regulatory components of genes to enhance or inhibit the activity of specific genes. Usually, the adjustment of these genes is introduced into cells through viral vectors or non-viral vectors, so as to directly interfere with genetic information and achieve therapeutic purposes.

In terms of cancer, gene therapy treats cancer by directly modifying or regulating the genes of cancer cells, fundamentally preventing their growth, promoting their death

or enhancing the immune system. It can introduce healthy genes into cancer cells, replace or repair mutated tumor suppressor genes, or use gene editing tools such as CRISPR-Cas9 to accurately delete oncogenes. In addition, gene therapy can also introduce suicide genes to make cancer cells self-destruct under the action of specific drugs, or modify immune cells to recognize and kill cancer cells more effectively. In these ways, gene therapy provides a new way to treat cancer at the genetic level.

2. Gene Therapy Strategies in Cancer

2.1 Suicide Gene Therapy

Suicide Gene Therapy (SGT) is a promising cancer treatment strategy that triggers self-destruction under specific conditions by introducing specific genes into tumor cells. The therapy mainly works through two mechanisms: one is the enzyme-prodrug system, taking the herpes simplex virus thymidine kinase (HSV-TK) gene as an example [2]. The enzyme encoded by this gene can convert non-toxic precursors, such as ganciclovir(GCV), into toxic metabolites, thus specifically killing tumor cells carrying the gene. The herpes simplex virus-thymus kinase (HSV-TK) gene/GCV system is one of the most widely studied tumor suicide gene therapies. For example, in the treatment of glioma, the HSV-TK gene is introduced into tumor cells through a viral vector, and then treated with GCV, which can accurately destroy tumor cells without damaging normal tissues [2]. Although it shows efficacy in rodent models, this effect has not been confirmed in clinical studies due to the low gene transduction efficiency of systemic drug administration and the lack of an effective gene delivery system. Research by Li et al. shows that radiofrequency hyperthermia (RH) can improve the efficiency of chronic virus-mediated gene transfection, especially the direct injection of high-dose HSV-TK gene in the tumor, which can improve the effect of gene transfection and improve its clinical application potential [3].

Another is the direct expression of toxin genes, which can destroy the key structure of tumor cells or activate their apoptosis signal pathways, leading to cell death [2]. Although suicide gene therapy is still in the clinical research stage, its potential in the treatment of many malignant tumors such as glioma and liver cancer has been widely recognized [2]. Future research will continue to focus on how to deliver genes more accurately, enhance treatment effects and reduce side effects, with a view to transforming this treatment into a conventional clinical treatment and providing patients with a more effective personalized treatment plan.

2.2 Tumor Suppressor Gene Activation

In normal cells, tumor suppressor genes play an important role in the normal growth and differentiation of cells and prevent the occurrence of cancer. The deletion of tumor suppressor genes or inactivation due to mutations can lead to cancer.

2.2.1 Rb gene

The Rb gene was the first tumor suppressor gene to be identified, and its major functional regulation includes inhibition of phosphorylation and activation of dephosphorylation. The Rb pathway regulates apoptosis factors by transcription regulation of apoptosis factors, and the activated E2F transcription factor induces apoptosis by transcription activation of apoptosis genes,E2F transcription factor is an indispensable partner in Rb gene research. Most cancers have inactivated Rb pathways, which is due to Rb mutation/missing or functional inactivation, and a small part of the remaining cancers, due to the need for functional Rb, may also have the over-expression or amplification of Rb. Therefore, using the Rb pathway as a guide to treat cancer and using different specific targeted cancer cells according to its state has become a role of the Rb gene in cancer treatment. For cancers with inactivated Rb pathways, killing Rb defective cells is the most intuitive way. Model organisms like Drosophila can be used to identify genes that regulate the apoptosis of Rb mutant cells to help find drug targets. For cancers without inactivation of the Rb pathway, the development of small molecule inhibitors with Rb function may be helpful [4].

For cancers with reversible inactivation of Rb pathway, it can be prevented by reactivating the Rb function. The activation of Rb genes usually requires the mediation of CDK4/6, resulting in the restriction of tumor cell proliferation to slow down disease progression. While the over-phosphorylated Rb-NF-kappa B axis can be used to support cancer immune escape induced by conventional or targeted therapies [5]. In addition, loss of the Rb gene has been found to be associated with drug resistance in targeted drug therapy, which provides a basis and framework for continued Rb research in the future.

2.2.2 P53 gene

The P53 gene is also an important tumor suppressor gene. In recent years, it has been found that the defects of the P53 gene are very common in cancer patients. Gendicine (recombinant human p53 adenovirus) is a gene therapy that has been approved by the China Food and Drug Administration (CFDA) in 2003 and is relatively widely used in the treatment of head and neck cancer and other cancers [6]. It expresses wild- type p53 protein by transducing cells and triggers a cellular stress response, thus

promoting cell cycle stagnation, DNA repair, cell apoptosis, senescences or autophagy. In addition, treating cancer by activating or restoring the wild- type characteristics of p53 mutants expressed in cancer is also a treatment strategy that is now widely concerned. It has been found that such as some molecules (including PRIMA-1), zinc, third-generation ammonia thiourea (COTI-2) and so on have different degrees of functions for different types of p53 mutations in different directions, but this treatment strategy still needs to be further studied and verified [6].

2.2.3 CDK inhibitor

Traditionally, it is believed that in cancer is due to a disruption in the balance of the cell cycle. Cells receive too many mitotic signals, inhibitory checkpoint failure or both, which leads to excessive cell division. Based on this, the development of inhibitors of cyclin- dependent kinases (CDKs), which regulate the cell cycle, is thought to be one of the ways to treat cancer. After very poor clinical progress in the development of the first two generations of CDK inhibitors, CDK4 and CDK6 inhibitors optimized for the pyrido[2,3-d]pyrimidine scaffold by including a methyl substituent at the C-5 position, have excellent enough selectivity [7]. Since 2004, the chemical structure of the first CDK4/6 inhibitor, palbociclib, was published, several CDK inhibitors have been successfully investigated and approved bythe United States Food and Drug Administration (FDA), such as ribociclib and abemaciclib, also used in the treatment of breast cancer, and trilaciclib, used to reduce myelosuppression induced by chemotherapy in patients with small cell lung cancer (SCLC) [7]. Many other selective CDK4/6 inhibitors are currently in various stages of clinical development.

2.2.4 BRCA1/2

BRCA1 and BRCA2 are tumor suppressor proteins that have the effect of assisting in the repair of damaged DNA, or eliminating cells when DNA cannot be repaired.It is generally expressed in the female organs and thus its mutation plays an important role in the development of breast cancer. In addition to molecularly targeted therapies that have been approved by the FDA, therapeutic strategies that provide access to the wild-type BRCA gene have become an area of research for the treatment of breast cancer. These include Holt et al. 1996: Retroviral transfer of the wild-type BRCA1 gene impedes the growth of all breast and ovarian cancer cell lines tested in vitro [8]. And the study of Ibnat and Chowdhury showed that restoring BRCA1/2 tumor suppressor activity by intracellular delivery of BRCA1/2 gene with the help of CA NPs is able to delay the growth of breast tumors, which also provides a new therapeutic approach for breast cancer [8].

2.3 Immunotherapy

The immune system plays a key role in resisting the invasion of malignant cells. In recent years, cancer immunotherapy has completely changed the field of oncology and promoted the rapid development of cancer treatment strategies. These strategies include new treatments such as immune checkpoint inhibition, chimeric antigen receptor T-cell therapy and cancer vaccines. As an important part of the adaptive immune system, T cells have promoted significant progress in cancer immunotherapy because of their ability to recognize and eliminate cancer cells. In the mid-20th century, Thomas and Burnett first proposed the concept of lymphocytes as an anti-tumor monitoring medium, and T cells were later identified as the main mediator of adaptive immunity [9]. Today, the role of T cells in cancer immunotherapy has become a rapidly developing research field. Scientists are trying to find ways to get T cells to recognize cancer cells. One possible way to do this is CAR T-cell therapy. CAR T-cell therapy is a type of immunotherapy.

CAR stands for chimeric antigen receptor. Chimeric antigen receptor (CAR) is a synthetic receptor used to redirect lymphocytes to recognize and destroy specific target cells. The CAR consists of four main components: an antigen-binding domain, a hinge, a transmembrane domain, and an intracellular signaling structural domain, each with a unique function [10]. By altering these structural domains, the design of the CAR can be optimized. CAR T cells have become an important treatment for relapsed and refractory B cell lymphomas, B cell acute lymphoblastic leukaemia and multiple myeloma. Although anti-CD19 CAR-T therapy has achieved remarkable results in the early stage, it also faces the problems of high relapse rate and drug resistance, which promotes the development of more effective CAR-T cells [9]. The innovation of CAR-T cell structure and manufacturing, especially the development of fourth-generation CAR-T cells, has significantly improved the efficacy and durability [9]. The fourth and next generation of CAR-T cells, combined with immunomodulators, have become an effective tool to overcome the tumor microenvironment.

At present, The CD19 targeted CAR-T cell products approved by the FDA include axicabtagene ciloleucel, tisagenlecleucel, lisocabtagene maraleucel and Brexucabtagene autoleucel [11]. Tisagenlecleucel was the earliest approved for initial approval for recurrent or refractory (r/r) children and young adults with B-cell acute lymphocytic leukemia (ALL), and later approved for adult r/r diffuse large B-cell lymphoma (DLBCL) [11,12]. Axicabtagene ciloleucel is used to treat adult large B-cell lymphoma that is ineffective for more than two other therapies, in-

cluding DLBCL and other types [12]. In April 2017, the FDA also approved it for recurrent or refractory DLBCL and awarded it breakthrough therapy recognition [12]. In 2021 and 2022, two BCMA-specific CAR T-cell therapies idecabtagene vicleucel and ciltacabtagene autoleucel have also been approved for the treatment of multiple myeloma [11,12]. These products express CAR through virus transduction of patient's autologous T cells and use second-generation constructs, including antigen-binding domains, hinges, transmembrane domains, co-stimulus domains (from CD28 or 4-1BB) and T cell activation domains (from CD3ζ) [13]. Although the specific domain, viral vector and manufacturing process of these products are different, lymphocyte removal chemotherapy is performed before CAR-T cell infusion to support the proliferation and activation of CAR-T cells [13]. Approved CAR-T cell products can cause some specific adverse reactions to varying degrees, including cytokine release syndrome (CRS), immune-effect cell-associated neurotoxic syndrome, hypogammaglobulminemia and cytopenias, especially B cell dysplasia [13].

2.4 Inhibition of Oncogene Therapy

Targeted therapies based on RNA interference (RNAi) and antisense oligonucleotides (ASO) are developing rapidly, selectively targeting RNA in a sequence-dependent way to treat genetic diseases and cancer. This kind of therapy is often referred to as inhibition of oncogene therapy, which refers to any gene therapy that can change the coding and non-coding RNA levels in the transcriptionally or post-transcriptionally to achieve therapeutic results. Commonly used tools in transcription regulatory therapy include RNAi and ASO. ASO is composed of 12-28 base, short-length single-stranded deoxynucleotides, which can complement and bind to the target mRNA or pre-mRNA sequence to form DNA/RNA heterologous double-strands [14]. Through this combination, ASO can regulate the expression of target genes.

In cancer treatment, the combination of ASO and transcription can prevent the ribosome loading of RNA, inhibit the translation of mRNA, and thus prevent the expression of oncogenes. For example, ASO is designed to target and downgrate the KRAS gene, including its wild and mutant types, to inhibit signal transmission and proliferation in cancer cells [14]. These ASOs, such as AZD4785, have shown anti-proliferative effects in lung cancer and colon cancer cell lines. ASO inhibits tumor angiogenesis and cell proliferation by blocking the expression of hypoxia-inducing factor-1α (HIF-1α), thus inducing tumor cell apoptosis. Although its systemic side effects limit some clinical applications, the improved conditions ASO show better specificity and safety [14]. ASO is also used to reduce the expression of heat shock protein 27 (Hsp27) to enhance the sensitivity of cancer cells to chemotherapy drugs, especially in the treatment of bladder cancer and pancreatic cancer [14]. Clinical trials show that this treatment can prolong the survival of specific patients.

Sometimes ASO is also used with other treatments to get better results. For BRCA-mutated ovarian cancer, the FDA has approved the use of PARP-1 inhibitor, olaparib, an enzyme that is involved in DNA single-strand rupture (SSB) repair and DNA replication [14]. PARP-1 inhibition caused by olaparib will induce replication fork stagnation, resulting in double-chain fracture (DSB), resulting in replication failure [14]. However, it is only effective for HRR defective cells, while HRR defective cells are resistant to drugs. A preclinical study showed that the combination of antisense oligonucleotides for BRCA2 and olaparib can make many human cancer lines sensitive to the drug, thus increasing the incidence of chromosomal translocation and aneuploidy, and blocking various tumor cell groups from taking olaparib (and generally against PARP) -1 inhibitor) produces drug resistance [14]. ASO is used to silence FoxP3 expressed by regulatory T cells (Treg), thus preventing immunosuppression in the tumor microenvironment [14]. When combined with cancer vaccines, this treatment strategy shows the potential to delay tumor growth and improve survival. ASO is also used to target eukaryotic translation initiation factor 4E (eIF4E) to prevent tumor-causing transformation of colorectal cancer and other cancer cells by inhibiting its overexpression. Although ISIS 183750, used in combination with Iliticon in clinical trials, failed to significantly improve the therapeutic effect, but it showed certain clinical activity in specific patients [14]. ASO (such As BP1001) targeting the Grb2 protein inhibits cancer cell proliferation by preventing its overexpression In acute myeloid leukemia and chronic myeloid leukemia. Clinical trials show that BP1001 combined with traditional treatment can improve the survival rate of patients [14].

2.5 Antiangiogenic Gene Therapy

Anti-angiogenic therapy is an ancient anti-cancer method that aims to fight cancer by inhibiting the formation of new tumor vascularization. Because tumors need these new blood vessels to provide oxygen and nutrition to support their growth and diffusion, anti-angiogenic therapy aims to "starve" the tumor by cutting off these supplies, thus slowing down its growth and may cause it to atrophy. Although different molecular media play an important role in controlling cancer angiogenesis, vascular endothelial growth factor (VEGF-A, also referred to as VEGF) is the most in-depth and targeted cancer treatment so far. Most antiangiogenic drugs approved for cancer treatment rely

on the effect of vascular endothelial growth factor (VEGF), because VEGF signaling is considered the main angiogenesis promoter. In addition to controlling angiogenesis, these drugs can also enhance immunotherapy, because VEGF also has immunosuppressive functions. However, the treatment of single-targeted VEGF often faces drug resistance, which may be due to the activation of other signaling pathways and the increase of angiogenesis factors. For example, after VEGF is inhibited, the fibroblast growth factor (FGF) pathway may be activated to enhance angiogenesis [15]. The use of inhibitors targeting VEGF and FGF, such as dovitinib, can delay tumor growth. In addition, angiopoietin (ANG) also plays a role in VEGF inhibition of drug resistance, and the treatment of double blocking VEGF and ANG2 can inhibit vascular reconstruction. Hepatocyte growth factor (HGF) increases expression after VEGF inhibition through its receptor c-MET, which promotes angiogenesis and tumor resistance [15]. Through multiple-targeted VEGF and new therapies with its alternative pathways, it is expected to overcome drug resistance and improve the effect of cancer treatment [15]. Although people have studied the anti-VEGF strategy for a long time, its clinical performance is far less than that of preclinical success, the most serious of which is the safety problem [15].

In order to overcome the problem of insufficient anti-VEGF therapy, researchers have also tried to study many alternative methods, and endostatin is preferred by some researchers. Endostatin is one of the most studied peptides with angiogenesis inhibitory. Endostatin is an effective angiogenesis inhibitor composed of C-terminal lysis fragments of type XVIII collagen [16]. Although its specific mechanism of action is not completely clear, studies have shown that endostatin inhibits angiogenesis in a variety of ways, including interfering with extracellular matrix elements and preventing the migration and proliferation of endothelial cells. Recombinant human endostatin enhances its stability and anti-angiogenic effect by adding additional amino acids. At present, researchers have studied different types of cancers such as lung cancer, stomach cancer and esophageal cancer, and found that it is also effective in the treatment of cancer. However, due to the short research time and insufficient data, it is impossible to prove that endostatin can be a substitute for anti-VEGF [16]. However, endostatin has reflected its potential, especially compared with anti-VEGF therapy, endostatin is safer.

3. Conclusion

At present, most clinical studies are still in the initial stage, gene therapy has not completely replaced traditional therapy. Although pure gene therapy, like inhibition of oncogene activation, has not yet achieved the expected results, it is being used in combination with existing treatment methods. Among them, suicide gene therapy's combination with chemotherapy shows huge promise. By injecting genetic material directly into the tumor, the suicide gene can effectively reduce the side effects and is compatible with existing treatment methods. Gene therapy provides a new way to treat and prevent various complex cancers. Unlike traditional treatment methods, gene therapy focuses on the root cause of the disease. By modifying or replacing defective genes, it not only alleviates or alleviates the disease, but also focuses on correcting the disease from the source. At the same time, it can reduce the dependence on long-term drugs to save the cost of long-term treatment, and improve or avoid drug resistance in cancer treatment and damage to physical health cells and tissues. The development of gene therapy has also promoted scientific research in gene editing, virus vector design, immunology and other fields, laying the foundation for future medical innovation. Although there are still many gaps in people's research on gene and gene therapy, gene therapy has shown great potential in the field of cancer treatment, especially in the treatment of refractory or recurrent cancer. We hope that through the continuous exploration and clinical trials of researchers, we can see more specific, clear, effective and even personalized gene therapy. We hope that through the continuous exploration and clinical trials of researchers, we can see more specific, clear, effective and even personalized gene therapy. Overall, gene therapy represents a major breakthrough in the medical field. Although it still faces technical and ethical challenges, it is expected to become the most important tool for cancer treatment in the future.

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