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The Use of Bacteriophages in Cancer Therapy

Alireza Gharavi[1*]

[1]Department of Nanobiotechnolog Biomimetic, University of Tehran, Iran.

Corresponding Author's E-mail: gharavial@gmail.com.

Abstract

Cancer is a catastrophic illness with a significant worldwide fatality rate, anticipated to rise in the next years. Contemporary treatment modalities, including chemotherapy and radiation therapy, include constraints such as adverse effects, inconsistent efficacy, elevated expenses, and restricted accessibility. Bacteriophages have arisen as multifaceted instruments in bioengineering, with significant promise in tissue engineering, vaccine formulation, and immunotherapy. Bacteriophages are being used extensively in several fields of biotechnology and medicine, with cancer treatment being the most compelling application. A multitude of research is progressively confirming the efficacy and efficacy of phage-based carriers as broad delivery mechanisms for medicinal genes and medications in cancer therapy. Furthermore, the genetic makeup of phages may be utilised in the development of novel DNA vaccines and antigen presentation systems, since they offer a highly organised and repetitive presentation of antigens to immune cells. Bacteriophages have generated new possibilities for the accurate targeting of particular molecular markers in cancerous cells. Phages may function as anticancer agents and as vehicles for imaging agents and pharmaceuticals. This article presents bacteriophage and analyses the efficacy of bacteriophages and bacteriophage engineering in specific cancer treatment.

Keywords: Bacteriophage, Cancer treatment, Cancer vaccine, Phage display.

Introduction

In the last century, understanding of the biology of cancer has markedly progressed. This progress has been substantially propelled in the past few years by technological innovations and concepts across multiple fields, such as next-generation sequencing, "omic" sciences, high-resolution microscopy, molecular immunology, flow cytometry, individual cell analysis and sequencing, novel cell culture methodologies, and the creation of experimental animals, among others. Nevertheless, several problems remain unresolved and many difficulties continue to exist about this illness (1). Consequently, oncological research is deemed essential. Cancer now ranks among the leading causes of mortality globally. Data provided by the World Health Organisation (WHO) in 2020 indicates that cancer is the second leading cause of death globally, resulting in 10 million fatalities. Cancer is a predominant global issue (2). Cancer is a pathological condition that may manifest in several anatomical locations within the body of a person, such the lungs, breasts, male reproductive system, and colon, among others. Numerous factors correlate with an increased likelihood of cancer onset, including age, genetic susceptibility, lifestyle decisions such as tobacco and alcohol use, and exposure to environmental toxins. However, not all cases of the disease can be attributed to known risk factors, and some individuals may get malignancy without any apparent cause. The incidence of cancer is increasing; yet, the variability of lesions and their aetiologies complicates the

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development of personalised therapy for each patient. Surgery a resection treatment with chemotherapy, and radiation treatments have been the fundamental approaches of cancer treatment for years. While surgery is the preferred method for eradicating primary tumours, there are situations when not all cancerous cells are removed, resulting in tumour recurrence (2). Chemotherapy and radiation therapy can cause considerable cytotoxicity in normal cells, making them inappropriate therapeutic options for some types of cancer or individuals. Conventional treatments are incapable of specifically eliminating cancer cells while safeguarding healthy cells and tissues. Several factors must be assessed while developing cancer-targeting treatments, notably the detrimental microenvironment that facilitates the development of tumour cells. The study of cancer seeks to develop an effective therapeutic strategy using novel treatment and preventive methodologies. The tumour microenvironment is a vital component in the realm of immunology and this developing therapy paradigm. Moreover, the number of available medicines has increased owing to progress in gene editing and other biotechnological techniques (4).

Bacteriophages, or phages, are a diverse group of viruses with varying sizes and morphologies (icosahedral forms like λ, T4, T7, and filamentous types such as fd, f1, M13) that infect bacteria and lyse host cells to produce progeny phages for further infections, while typically not infecting eukaryotic cells (3, 4). Phages are the most abundant class of microorganisms in the biosphere and contaminate almost all identified pathogenic bacteria. Twort and d'Herelle separately discovered bacteriophages in 1915. Twort seemed to support the notion that it was not a distinct living form, but rather an enzyme released by bacteria. He forsook phage research and dedicated the remainder of his scientific career to the production of animal viruses. Nonetheless, d'Herelle first introduced the name bacteriophage and dedicated his life to the study of bacteriophages and their therapeutic applications in humans and animals (6). The utilisation of phages in anti-cancer or anti-tumor therapies represents a novel domain. The latest developments indicate that phage research has progressed to its second generation.

Phages used in cancer treatments and assessment as selective nanocarriers as and in gene therapy as carriers for therapeutic DNA or RNA. Phages are studied in immunological vaccine research due to their capacity to provoke both cell-mediated and antibody-mediated responses (7). Gene insertion may facilitate the production of specific peptides or protein fragments inside the phage capsid, therefore presenting them to immunological regions to provoke a vigorous immune response against diverse antigens from cancerous cells. Due of its small and uniform size, it is the most effective nanoparticle for drug delivery and multiple other uses, such phage presentation and focussing (6). Their omnipresence in nature allows their capsids to elicit an immune reaction or, through genetic or protein modification, enable any organism to produce specific proteins on its outermost layer to activate an immune system reaction. Another approach utilises phage display technology to generate recombinant humanised monoclonal antibodies that particularly target neoplastic cells and harmful microorganisms. Phages provide considerable promise for theragnostic applications, gene therapy, and immunotherapy in cancer therapy due to their nanoscale dimensions, flexible surface properties, precise target specificity, inherent safety, and non-pathogenic nature (8). This article highlights the promise of phage therapy as an innovative approach in cancer treatment. The book analyses the distinctive features of bacteriophages which offer feasible alternatives for targeted and customised cancer therapies.

Composition and biology of bacteriophages

Bacteriophages are essential bacterial parasites and represent the most prevalent group of biological creatures in the biosphere. In the lack of genes necessary for autonomously development, phages employ the biological machinery of host cells to produce their genetic material (6, 7). Bacteriophages demonstrate extraordinary variety and infected almost all referred to bacteria. These types of viruses substantially impact the evolution of prokaryotic species, modify bacterial interactions, and may disrupt the relationships between bacteria and multicellular organisms (9). The distinctive characteristics of bacteriophages make them a potential instrument in several domains of biotechnology. Furthermore, phages and bacterial anti-phage defence mechanisms have profoundly influenced the creation and advancement of genetic engineering. The distinctive architecture and genetic arrangement of bacteriophages greatly enhanced their attractiveness. Bacteriophage fragments have a distinct three-dimensional shape (9).

The primary structure of virions consists of a head and a tail. The head contains the virus's genome, which is enclosed by a capsid. The genetic information may include either one-stranded or two-stranded DNA or RNA. The baseplate, located at the tail's termination, is an essential element of the bacteriophage structure, into which elongated, thin filaments extend, facilitating the phage's attachment to bacteria. The baseplate contains receptor-binding proteins that recognise specific substances on the outermost layer of the bacterial membrane (10). The relationship between bacteriophages and cells of bacteria is specific; the target must possess the

requisite receptors on its membrane the outside, enabling phage attachment and internalisation. Certain bacteriophages are devoid of a tail. They possess receptor-binding proteins on the outside of their capsids. The interaction within a phage and a host bacteria occurs at many stages. Subsequent to the first interaction, enabled by the dispersion of phage fragments in the fluid and Brownian motion, electrostatic interactions promote reversible and unspecified adherence of the phage to the bacterium. The attachment becomes irreversible due to the binding of viral capsid elements to cell surface receptors, which may consist of amino acids, teichoic acids, glycoproteins, lipopolysaccharides, or pili segments, contingent upon the phage type (11, 12).

The baseplate coordinates the recognition of particular receptors on the cell surface and the adhesion of the phage fragment to the host bacterium. The effective binding to the microbial receptor causes a conformational change in the baseplate, resulting in tail shortening. The catalyst for the onset of these events is a modification in the position of the fibrils in relation to the baseplate. The reorientation of fibrils occurs only on the outermost layer of the host cell and is undetectable in the free state of viral pieces in solution (13). Additionally, no chemical energy is used to modify the filament direction and baseplate configuration.

Various proposed mechanisms clarify the particularities of phage attachment to the host cell. The strong binding of phage to a microorganism is enabled by the partial attachment of viral fibrils to particular receptors on the cell appear, while the alignment of the whole virion on the cell surface and later interactions of fibrils with cell receptors are affected by the movement of the surrounding medium. The restriction of phage fragment mobility causes a conformational change in unbound fibrils, increasing their attraction for the cell membrane. Another proposed mechanism is that the modification of the three-dimensional configuration of the baseplate and the reorientation of the viral strands towards the cell, leading to the strong adhesion of the phage to the bacterium, is mediated by divalent cations, especially Ca2+ (12, 13).

Numerous membrane polysaccharides and proteins of bacterial cells engage with divalent cations, significantly increasing their concentration at the interface between the phage and the host. It has been shown that, for some bacteriophages, the existence of calcium ions is crucial for the infection of host cells. The efficacy of bacteriophage engagement with prokaryotic cells is influenced by the presence of additional ions in the solution; heightened ionic strength augments the infectious capacity of phage fragments (14). It is essential to acknowledge that these methods are not incompatible and may concurrently coexist. Subsequent to the irreversible binding of the phage to the bacterium, the contraction of the viral protein coat occurs, during which the rigid tail tube penetrates the outermost membrane of the cell. Thereafter, the tube penetrates the periplasmic space and, with the assistance of enzymes at its end (mostly lysozyme), selectively dismantles the peptidoglycan of the cell membrane. The viral genetic code is then transferred into the bacterium's cytoplasm, however the phage's protein coat remains exterior.

Regardless of the method by which phage genetic material enters the host cell, there are a couple possible techniques for its future implementation. At the commencement of the lytic cycle, a thorough rearrangement of cellular metabolism occurs; the cell's energy is wholly diverted to the replication of viral genetic material, the transcription of viral genes, and the synthesis of viral proteins. The last stage of this cycle is the assembly of matured virions and their next dissolution by cell lysis. The lysogenic cycle is characterised by the reversible incorporation of the phage DNA into the bacterial genetic structure (16). In this circumstance, the viral genome replicates simultaneously with the host's DNA, while maintaining cellular integrity. The lysogenic cycle mostly endures until the activation of the prophage and the shift to the lytic pathway occur. A comprehensive analysis of bacteriophage structure, how they interact with bacteria hosts, and their lifespan facilitates the application of these viruses in multiple areas of modern practical and theoretical research (17).

Due to the rising antibiotic resistance of bacteria, phage therapy for infectious diseases is now seeing a revival. The effectiveness of bacteriophage use in food-related diagnostics has been demonstrated. Phages are used in the control of plant diseases, biomedical diagnostics, biological research, and genetic engineering. Bacteriophages are considered potential vectors for gene therapy for cancer (15, 16). They are regarded as having several advantages compared to non-viral and eukaryotic virus-based carriers. Phage-based vectors are superior to eukaryotic viruses because they lack inherent tropism for eukaryotic cells, hence improving the specificity of therapeutic cargo being delivered to cancerous cells. Contrary to previous beliefs that phages could not interact with organisms more complex than prokaryotes, recent evidence has progressively demonstrated that natural bacteriophages may directly interface with the cell membranes of higher organisms (18).

Oncology treatment: present obstacles and innovative approaches

Bacteriophage-mediated gene therapy for cancer

Phage-mediated cancer gene treatment is a method

that utilises the capacity of phages to specifically deliver genes that treat to cancerous cells. Phages have been extensively studied and are employed in various fields, especially medicine. Traditional gene therapy often utilises viral vectors derived from mammalian viruses to deliver therapeutic genes into target cells (17). However, the application of mammalian viruses has safety concerns and limitations due to their ability to integrate into the host genome, which may lead to unpredictable consequences. Phages provide a viable option owing to their pronounced selectivity for bacterial hosts and their incapacity to infect human cells. In the 1940s, Bloch presented the first evidence of phages' capacity to directly engage with mammalian cells. His findings suggest that phages may aggregate in malignant cells, impeding cancer growth (18). Kantoch subsequently demonstrated that phages could attach to and penetrate guinea pig leukocytes. A new investigation has reinforced the established associations among phages and mammalian immune system cells (19).

Despite being considered ineffective carriers for generating higher organism cells, bacteriophages unexpectedly offered a unique strategy for creating therapeutic gene methods of delivery. Bacteriophages are seen as a promising approach for cancer gene therapy owing to their absence of a preference for human cells, enhanced cloning efficiency, simplicity of alterations, and use of phage technology for display. The production of phage fragments within bacterial cells is defined by efficiency, speed, and cost-effectiveness (20).

Phages may be modified to transport therapeutic genes targeting various objectives in cancer treatment. For example, they may encode proteins that induce apoptosis in cancer cells, inhibit tumour development, or enhance the immune response to cancer cells. Phage-based gene therapy offers the potential for targeted and precise treatment by directly administering therapeutic genes to cancerous cells, thereby decreasing off-target effects. Moreover, phages have inherent advantages as vectors for gene transfer (21, 22). They are straightforward to manipulate in the laboratory, and their ability to infect a broad spectrum of bacteria enables the development of a varied library of phage variations with unique tumor-targeting properties. Moreover, phages may be administered via several methods, including intravenous injection, targeted injection, or consumption, providing diversity in therapeutic approaches. Despite the significant promise of phage-based malignancy gene therapy, issues must be resolved. Additional efforts are necessary to improve phage design, optimise targeting efficiency, and ensure the safety and effectiveness of this method. Ongoing clinical investigations are assessing the therapeutic potential of phage-based gene therapy for several types of cancer (23).

Phages have been recognised as promising carriers for gene therapy in oncology via many research and clinical trials. Despite the prior preference for eukaryotic viruses due to their enhanced efficacy in generating mammalian cells, their natural a preference for eukaryotic host cells presents challenges for therapeutic applications. Retroviral and lentiviral vectors possess drawbacks, such as potential oncogenicity, restricted replication of the target gene, and significant immunogenicity associated with adenovirus-based vectors. These issues have impeded the progress of gene therapy methodologies for cancer treatment. Recombinant adeno-associated virus (AAV) carriers have potential owing to their efficacy; yet, they are limited by reduced packaging capacity, the existence of neutralising antibodies, and a requirement to enhance transduction selectivity for systemic administration. In contrast, phages possess unique architectural and biological characteristics that provide innovative methods for targeted transfer of genes to cancerous cells. Scientists are exploring the possibility of phages as gene therapy carriers, therefore overcoming the drawbacks of other viral vectors. The effectiveness of the AAV/phage (AAVP) vector in suicide gene therapy for malignancy has been reliably corroborated in subsequent studies. AAVP-RGD4C-HSVtk, in combination with GCV, exhibited a substantial antitumor effect in preclinical trials utilising mouse models of Kaposi's sarcoma, bladder and prostate carcinoma, breast tumours, nude rats with human sarcoma xenografts, rat glioblastoma cells, mouse simulates of human glioblastoma, and human melanoma tumour cells (21, 24, 25).

Phage-based vaccines

Phages may be engineered to deliver specific antigens to the immunity system for vaccination purposes. Antigens are substances that elicit an immune reaction, leading to the production of antibodies and the formation of immune memory. Vaccines educate your immune system to recognize and combat certain diseases, particularly infections caused by bacteria and viruses, by introducing antigens. The use of phages as a vehicle for antigen transport in vaccinations provides multiple benefits: Phages has an innate ability to spread disease and target certain bacteria according to their intrinsic affinity. Phages may be modified to present antigens on their appears allowing them to selectively target certain bacteria and enhance the body's defense against particular germs; (ii) Phages possess significant immunogenicity owing to their inherent immunogenic characteristics. They may proficiently stimulate immune responses that are innate as well as adaptive when employed as a vaccine delivery strategy (30, 31). This may elicit a robust immune

response to the given antigens; (iii) Enhanced stability; phages demonstrate exceptional resistance to external conditions, including variations in temperature and pH levels. This stability makes them attractive candidates for vaccine development, since they can withstand the challenges of storage, transportation, and distribution. The manipulation of phages is very simple owing to their susceptibility to genetic alteration. Researchers may integrate genes encoding specific antigens into the phage genome, resulting in the display of these antigens on the phage membrane. This genetic engineering enables the precise development and customization of phage-based vaccines, as well as the potential for combinatorial immunizations, allowing phages to simultaneously deliver several antigens. This characteristic facilitates the creation of combination vaccines that target many illnesses or strains in a single formulation. This approach may optimize immunization schedules and improve overall vaccine acceptance (32).

The development of vaccines has extensively utilized multiple lytic and filamentous phages owing to their advantageous characteristics. Filamentous phages, especially those of the Inovirus family, such as M13, fd, and f1 phages, are favored due to their simple capsids, rod-like structure, and one-stranded (ss) DNA genome (33). These phages primarily infect microbes and has a historical history in phage visualization technology. The preference for phages in vaccine research is driven by the availability of established components and techniques for phage manipulation, particularly in the selection and production of antigens and antibodies. Recently, a wider array of phages, such as tailed phages including T4, T7, and λ, with icosahedral phages like Qβ and MS2, has been utilized in phage visualization vaccination methods for antigen presentation. Tailed phages, unlike filamentous phages, enable the production of larger peptides and proteins with more complex conformations. Filamentous phages predominantly exhibit short peptides (33).

Phage display technology offers a reliable approach for detecting surface markers on cancer cells and for creating effective anticancer peptides for therapeutic purposes. Phage display vaccines may generate tailored immunogenic viral fragments by attaching antigens to phage membrane proteins. A multitude of applicants have been evaluated as phage-based cancer vaccines in preclinical studies, incorporating epitopes from the VEGFR2, EGFR (34), HER2, MAGE (35), MUC1 (36), FGFR, Flt4, and mimotopes of TAA. Multiple anticancer phage vaccines have been utilized effectively in immunotherapy against cancer (37).

VEGFR2 is highly expressed in tumor endothelial cells and functions as a cancer-associated antigen. A VEGFR2-targeted vaccine was developed using phage display technology, which generated anti-VEGFR2 antibodies that inhibited tumor progression in mice via CD4+ T cells (38). The application of T4 phages as a vaccine carrier may assist in overcoming immunologic resistance to VEGFR2. The delivery of T4 transgenic phages producing the extracellular region of VEGFR2 reduces VEGF-mediated tumor angiogenesis by selectively binding to VEGF, hence blocking downstream signaling cascades and diminishing tumor development and microvascular frequency in vivo (39). Monoclonal antibodies, such as bevacizumab, and peptides discovered using phage display technology have been designed to obstruct VEGF-dependent tumor angiogenesis (40).

Investigation has examined the application of EGFR peptide ligands as efficacious therapeutics for targeting overexpressed EGFR receptors in various tumor cell types. The phage display approach has been used to identify high-affinity peptides and antibodies that attach to EGFR, so blocking its signaling pathway and consequently decreasing tumor cell growth and viability (41). Phage-derived ligands demonstrate potential in the development of tailored therapies for EGFR-positive cancers. Distinct study revealed that antagonistic anti-EGFR nanobodies, selected by phage display technology, effectively obstructed the interaction of endothelial growth factor (EGF) with EGFR, impaired EGF-mediated signaling, and delayed tumor development in vivo. Phage exhibition screening via panitumumab-isolated EGFR mimotopes (P19 and P26) and HSP70-P19/P26 fusion proteins reduced tumor progression in lung cancer models, indicating the possibility for anti-EGFR therapy (34, 42).

DNA vaccines of phages

DNA vaccines provide considerable benefits over proteins or peptides immunizations due to their accurate antigen packaging and lack of subsequent processing demands. However, comprehensive primate investigations revealed insufficient immunogenicity, leading to the inclusion of adjuvants in human trials. The carriers must also address the instability and dispersion challenges linked to naked DNA vaccines (43, 44). In contrast, novel nucleic acid-based vaccines have demonstrated promising results in clinical experiments, particularly after the COVID-19 pandemic. Phage fragments possess adjuvant properties and function as an efficient method for DNA transfer. Bacteriophage DNA vaccines use eukaryotic expression cassettes using target-specific bacteriophage amplifiers that contain antigen-encoding genes. Phage DNA vaccines offer an acceptable substitute to naked DNA immunizations, with various advantages, including the ability to include substantial DNA antigens of

up to 20 kb. Lambda phages have been extensively studied as carriers for delivering DNA vaccines that encode proteins such as green fluorescent protein (GFP) and hepatitis B surface antigen (HBsAg), via cytomegalovirus (CMV) promoter-regulated reporter genes (20, 45).

They serve as a cost-effective gene delivery mechanism while also offering distinct advantages in nucleic acid immunization and in directing antigen-presenting cells to enhance immunity. Research suggests that lambda-ZAP E7 bacteriophage-mediated DNA transfer may efficiently deliver and produce curative genes, resulting in substantial anti-tumor advantages in immunized mice (46). However, DNA vaccines targeting Δ16HER2, a protein linked to the severity of breast cancer and resistance to treatment, failed to elicit immune protection in mice due to tolerogenic mechanisms. This challenge was mitigated by engineering bacteriophages with immunogenic epitopes of Δ16HER2, that provoked an immune anti-Δ16HER2 response, hence breaking immunological tolerance. These findings support phage-based anti-HER2/Δ16HER2 immunization as an efficient and secure treatment for HER2-positive breast tumors (47). Unlike peptide vaccination, lambda phage-based genetic vaccination induced immune system reactions marked by heightened antibody levels, augmented generation of cytokines, and improved epitope binding facilitated by a Th1 reaction.

Filamentous phages are under examination as vaccine carriers, enabling immunization with several epitopes or antigens through a singular delivery method (48). Furthermore, phage DNA vaccines are safe, uncomplicated, and economical, lacking antibiotic-resistance genetic material, and may be administered in many doses. They demonstrate durability and improve protein folding, contain the adjuvant characteristics of phage parts, and contain lipopolysaccharides and lipids, making them more effective than traditional DNA vaccines. Current literature lacks investigation on RNA-based phage vaccines for immunotherapy for cancer. To impede viral replication and deliver antiviral agents for myocarditis, study used synthesized microRNAs linked to folate-conjugated bacterial phage packaging RNA (pRNA). Thus, phage DNA vaccines offer a feasible approach for developing secure and efficient immunizations against many diseases (49).

Combination treatments and targeted oncological therapy

Targeted drug administration is becoming recognized for improving chemotherapeutic efficacy and minimizing its negative effects. Phage libraries can identify peptides that bind to cancerous cells, enabling targeted drug delivery to tumor sites. Peptides generated via phage presentation are frequently employed for tumor targeting because of their small size and ease of integration with drugs and carriers. Innovative methods for improving chemotherapy are being examined, with promising outcomes. Phages have demonstrated the ability to improve chemotherapy effectiveness while reducing its negative impacts (50). Scientists utilized M13-based phage collections to identify specific peptides that may traverse cell membranes and transport active pharmaceuticals to cancerous cells. Two peptide motifs, LTVSPWY and WNLPWYYSVSPT, have been found to bind to breast cancer cells and promote the internalization of antisense oligonucleotides. The AGKGTPSLETTP motif from a 12-mer M13-displayed phage library demonstrated notable anticancer efficacy in mice with hepatocarcinoma (HCC) tumors when co-administered with doxorubicin (DOX) (51). A pentapeptide phage library identified the ASSHN motif, to inhibit tumor angiogenesis; when combined with DOX-loaded liposomes, it exhibited significant growth inhibition in comparison with untargeted liposomes (52).Bacteriophages have been explored as vehicles for medications and diagnostic dyes, with favorable outcomes. M13 fragments displaying epithelial growth factors may effectively contain plasmids that express siRNAs targeting focal adhesion kinases, especially aimed at lung cancer cells (53). An anti-prostate-specific membrane antigen (PSMA) antibody was coupled with the gp3 protein to create an anti-PSMA-M13-SWNT system that preferentially addresses prostate cancer cells and is suitable for in vivo fluorescence analysis (54). Bacteriophages are employed in photodynamic therapy for oncological treatment. Studies demonstrate that MS2 bacteriophages decorated with DNA aptamers and M13 bacteriophages targeting breast cancer cells may efficiently deliver photosensitizers, resulting in cellular death (55). Phage-based nanotechnology via light therapy may address cancer by producing singlet oxygen, and studies suggest that genetically modified phages targeting SKBR-3 cancer cells induce cell death upon laser activation (56). An M13 phage, adorned with silver nanoparticles and engineered to attach to Fusobacterium nucleatum (Fn), was developed to specifically target Fn in colorectal cancer, leading to improved longevity in an animal model of orthotopic colorectal cancer (CRC) (57).

Progression of phage application in clinical investigations

Phage-displayed antigens offer advantages over traditional immunizations in stimulating the proliferation of T cells and enhancing the optimal immune reaction. Traditional vaccinations via soluble foreign antigens or inactivated pathogens do

not effectively stimulate T cells via the MHC class I pathway, resulting in inadequate immune reactions (58). Fibrous phages efficiently activate the MHC class I and II processes, essential for anti-cancer and anti-viral therapies (59). Phages may activate antigen-presenting cells to secrete costimulatory molecules, so stimulating T cells and positioning themselves as potential enhancers of the immune system's function (60). Phage therapy is now being evaluated in global research studies as a potential remedy for antibiotic-resistant bacterial infections in people. The American Clinical Trials database includes multiple articles about the use of phages in medical studies, mostly focused on the treatment of infectious diseases. Nonetheless, a lot of study has focused on the lytic properties of phages to combat antibiotic-resistant bacterial infections. In recent years, scientists have investigated the application of phages as a potential cancer treatment. A European phase I/II clinical trial has been conducted with patients receiving a phage-based vaccine that links a particular B-cell receptor to the exterior of phage fragments. The vaccination was assessed in patients with terminal-stage multiple myeloma and was well tolerated, with minimal and transient side effects. The immunization decreased blood paraprotein and urine-excreted myeloma-specific light strand stages, suggesting a therapeutic response in most individuals (61).

Notwithstanding promising results in preclinical studies, multiple obstacles have to be resolved prior to the widespread use of phage therapy in human cancer treatments. A multitude of clinical trials is under underway to evaluate the safety and efficacy of phage therapy in patients. ABNCoV2, a vaccine using virus-like particles (VLPs) produced from bacteriophage AP205 and decorated with the receptor-binding region of SARS-CoV-2, was synthesized in S2 Drosophila cells and then administered to healthy volunteers for safety evaluation in the clinical research (NCT04839146). Despite promising preclinical results and the efficacy of phage-based vaccines for humans and animals, the FDA and EMA have not yet granted clearance for these vaccines. The FDA has approved the application of bacteriophages as antibacterial agents in food products to combat contamination, namely Listeria monocytogenes in ready-to-eat meat and poultry (71 FR 47729). Moreover, several patents related to phage administration for cancer treatment have been granted or are under approval. The inventions involve multiple facets of phage therapy, such methods for phage manufacture and administration, specific phage formulations designed to target tumor cells, and strategies to employ phages to enhance the antitumor effectiveness of the body's immune response.

Constraints and prospective developments

Phage-based cancer treatment holds considerable promise as a secure, efficient, and personalized therapeutic approach. However, multiple constraints and issues must be addressed to fully harness the power it has. A significant limitation is the potential for immunological responses directed against the phages, which may reduce their efficacy and limit their use in persons with pre-existing immune impairments. Additional research is crucial to clarify the mechanisms of phage-induced immune reactions and to develop strategies for their reduction, such the use of mutant phages with less immunogenicity (62). Another risk is the emergence of immunity to phages, that might reduce their long-term efficacy. Bacteria may evolve and develop mechanisms for resistance against phages, similar to their reaction to antibiotics. Addressing this issue requires continuous monitoring and adjustment of phage formulations to surpass bacterial resistance processes. Furthermore, improving the display of antigens on phage surfaces for phage-based immunizations presents an additional challenge. This involves determining the most effective approach for presenting cancer-specific antigens on phages to provoke a robust immune response against cancer cells (63).

Ensuring safety and efficacy is paramount for phage-based therapies. Comprehensive preclinical and clinical investigations are crucial for accurately evaluating the potential detrimental effects and therapeutic benefits of phage treatments. The extensive production of phages is a prospective advancement that merits attention. To guarantee the extensive accessibility of phage therapy, it is crucial to develop economical and scalable production methods to meet treatment requirements. Moreover, regulatory clearance is a substantial barrier that phage-based therapies must overcome (64). Cooperation among researchers, clinicians, and industry stakeholders is essential to enhance the regulatory process and substantiate the safety and efficacy of these novel pharmaceuticals. Despite these limitations and challenges, additional study and investment in phage-based cancer therapy are crucial for unlocking its full potential. Combining the benefits of conventional cancer medicines with the targeted and personalized advantages of phage therapy may provide better, less harmful, and cost-efficient cancer treatment options in future decades (64).

Discussion and conclusion

Cancer is a significant global health issue, resulting in millions of deaths each year. The limitations of current therapeutic modalities, such as side effects, costs, and variable effectiveness, highlight the urgent need for more targeted and alternative treatments to improve patient results and standard of life. Phages

have become appealing agents in cancer therapy due to their high specificity, enabling targeted delivery to tumor cells while safeguarding healthy cells (65). This precision facilitates the mitigation of detrimental side effects linked to traditional treatments such as radiation therapy and chemotherapy. Furthermore, phages may be genetically modified and tailored, enabling individualized treatments that address specific cancer alterations or genomic irregularities in individual patients, hence potentially enhancing therapeutic efficacy and reducing the likelihood of resistance. Furthermore, phages have potential as nanocarriers for the administration of medicinal agents and as vectors for gene therapy. Their use in vaccine development to stimulate defenses versus cancer cells is also encouraging. Current investigations into phage-based therapies may revolutionize cancer treatment and offer hope to patients worldwide (66).

Cancer treatment has advanced significantly, including several conventional modalities like radiation therapy, chemotherapy, surgery, immunotherapy, specific therapy, and more. Each has persistent limitations, such as negative impacts, resistance, and cost. Phage therapy has attracted attention as a feasible alternative due to its specificity, flexibility, and genetic modifications, enabling targeted delivery to cells with cancer while sparing healthy ones. Its safety profile, ability to reduce toxicity, and potential to penetrate the tumor microenvironment make it a promising contender for future cancer therapies. Tailoring treatment to specific cancer types or mutations enhances efficacy, and its immune-modulating characteristics may further bolster the body's ability to fight cancer (67).

Phages are employed in nanotechnology for identifying and treating illnesses, healing of tissue, identification of bacterial and fungal infections, vaccine formulation, and gene therapy. Phages may be employed in personalized medicine for specific purposes. Phage-based vaccinations provide benefits over traditional immunizations in stimulating T cells and generating an optimal immunological reaction; nonetheless, their immunogenicity need enhancement prior to application. Phage display technology may be utilized in immunology of tumors for preventative and/or therapeutic vaccination or as a small-molecule drug. Phages may be engineered to function as more effective and precise vehicles for delivering drugs to cancerous cells, making them an attractive option for cancer treatment and testing, supplanting vectors derived from eukaryotic viruses. Phage treatment has shown efficacy in preclinical trials when combined with other drugs, enzymes, or particles. Scientists must address many challenges, include clarifying the mechanisms of phage interaction with the immune system and other cellular components, as well as confronting issues like phage resistance, allergic reactions, and other adverse consequences.

Bacteriophage-mediated cancer therapy has repeatedly shown its promise in several preclinical investigations to far. An rise in studies using phage-based vectors for the precise delivery of therapeutic transgenes into target tumor cells is envisaged in the near future. The altered phage particles offer a safer and more accurate systemic method for delivering therapeutic agents to cancer cells compared to carriers developed from eukaryotic viruses. The quest to improve the effectiveness of cancer gene therapy with bacteriophages has resulted in the development of innovative vector systems and transformational particles that demonstrate significantly greater amounts of targeted genetic expression in eukaryotic cells. Such vehicles may improve experimental methods for treating cancer by delivering therapeutic genetic information. The amalgamation of advanced vectors with CRISPR/Cas9 genetic modification technology constitutes a feasible approach for cancer therapy using genes. Enhanced improvement of this technology might lead to the creation of controlled nanoparticles demonstrating exceptionally specific nuclease activity aimed at eliminating mutant oncogenes.

Authors's Contribution

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