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REVIEW

Filamentous Phage for Therapeutic Applications in Non-Small Cell Lung Cancer and Brain Metastases: Recent Prospect

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Abstract: Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, with brain metastases occurring in 24% to 40% of advanced NSCLC patients and a poor prognosis. Traditional treatment methods for brain metastases, such as surgery and radiotherapy, often result in neurocognitive impairment and brain edema. Furthermore, chemotherapy drugs struggle to penetrate the central nervous system. Third-generation EGFR-TKI drugs that can cross the blood–brain barrier have demonstrated efficacy in treating NSCLC patients with brain metastases, but their benefits are limited to those with specific driver genes. Immunotherapy demonstrated potential in the treatment of NSCLC patients with brain metastases, although the adverse events limited its clinical use. Given these limitations, filamentous phages emerge as a promising bio-nanomaterial due to their unique biosafety profile, high solubility, and ability to facilitate targeted delivery, which can potentially minimize systemic toxicity. This review focuses on two core applications of filamentous phages in NSCLC and brain metastasis therapy: (i) phage display-derived targeting peptides and (ii) intact engineered filamentous phages as delivery scaffolds. As delivery systems, filamentous phages can prolong in vivo circulation time, reduce toxicity, and effectively cross the Blood–Brain Barrier (BBB)—evidences include filamentous phage mediating targeted delivery of chemotherapeutics and siRNA to NSCLC cells, and phage-nanomaterial hybrids enhancing tumor accumulation. The review also elaborates on the clinical translation potential of filamentous phages, including personalized therapy via patient-specific peptide screening, and discusses current limitations. Filamentous phage-based nanocarriers are expected to improve the quality of life of NSCLC patients with brain metastases.

Keywords: filamentous phage, lung cancer, brain metastases, nanoparticles, NSCLC

Introduction

Non-Small Cell Lung Cancer (NSCLC) is the most common type of lung cancer, accounting for approximately 85% of all cases, with major pathological subtypes including adenocarcinoma, squamous cell carcinoma, and large cell carcinoma.[1] Its occurrence is closely associated with driver gene abnormalities such as EGFR mutations, ALK fusions, and KRAS mutations. For instance, EGFR mutations are detected in 30–50% of Asian NSCLC patients, and these patients are more prone to brain metastases. Despite advances in treatment, the prognosis for NSCLC remains challenging, particularly in advanced stages: the 5-year overall survival rate of advanced NSCLC is less than 10%, and patients with brain metastases have a median survival of only 3–6 months.[2] According to the 2024 Global Cancer Statistics, NSCLC causes approximately 1.8 million deaths annually worldwide, with brain metastases being one of the leading causes of treatment failure.[3]

Traditional treatments such as surgery, chemotherapy, and radiation therapy have their limits, including resistance to therapy, severe side effects, and limited efficacy in patients with metastatic disease. Moreover, NSCLC patients with

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brain metastases face an even poorer prognosis, as the blood–brain barrier hinders the delivery of most systemic treatments. Although the development of targeted therapies (eg, EGFR-TKI, ALK inhibitors) and immunotherapies (eg, PD-1 inhibitors) has improved outcomes for certain patients subgroup, only 20–30% of patients respond to immunotherapy, and most EGFR-TKI-treated patients develop drug resistance within one year.[4,5] These limitations underscore the urgent need for innovative diagnostic and therapeutic strategies, particularly for managing NSCLC with brain metastases.

Filamentous phages, such as the M13 bacteriophage (Figure 1A), have emerged as a promising platform for both therapeutic and diagnostic applications for NSCLC and brain metastases. Unlike used to treat bacterial infection, these phages function as biological nanocarriers and molecular engineering tools for cancer therapy, leveraging their unique biological properties: 1) low immunogenicity and high biocompatibility: filamentous phages are non-pathogenic to humans, can be intravenously administered to patients without significant adverse effects;[6,7] 2) versatile engineering capacity: Their single-stranded circular DNA genome enables efficient insertion of therapeutic genes, while phage display technology allows construction of peptide libraries with $\geq 10^9$ unique sequences—critical for screening tumor-specific ligands;[8,9] 3) superior Blood–Brain Barrier (BBB) penetration: Their rod-like structure (diameter ~7–8 nm, length ~1 μm) and high aspect ratio enable non-toxic crossing of the BBB, a capability unmatched by most synthetic nanocarriers;[10,11] 4) multimodal payload delivery: filamentous phages can load chemotherapeutics, siRNA, cytokines, or nanomaterials via genetic engineering or physicochemical modification, supporting combination therapy.[8,12]

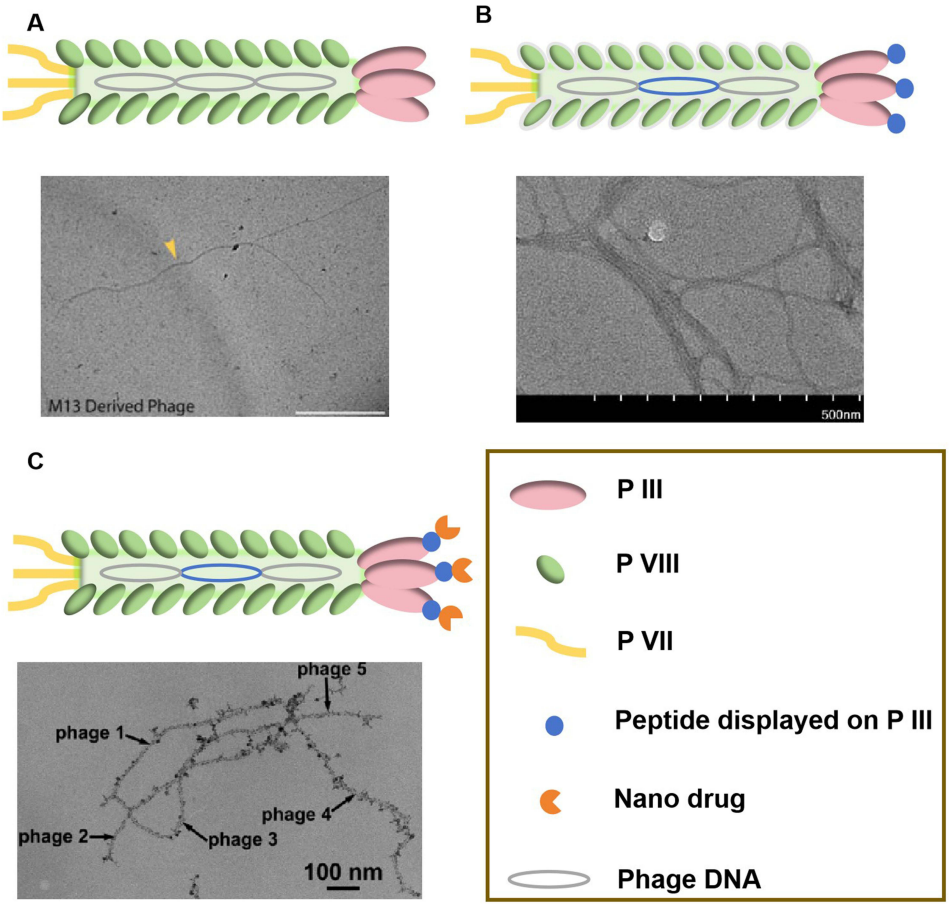

Figure 1 Schematic of phage drug carrier and its electron micrograph: (**A**) Wild filamentous phage (Scale bar =500 nm);[13](**B**) Peptide/protein drug phage carrier (Scale bar =500 nm);[14] (**C**) Nanomedicine phage carrier (Scale bar = 100 nm).[15]

This review focuses on the core applications of filamentous phages in NSCLC and brain metastasis therapy. We also elaborate on their clinical translation potential—including personalized therapy via patient-specific peptide screening—and discuss current limitations to provide a comprehensive overview of this emerging therapeutic platform.

Filamentous Phages: Biological Characteristics and Core Advantages

Biological Characteristics of Filamentous Phage

Bacteriophages have attracted extensive attention from biologists, chemists, material scientists and medical scientists because of their small genome, simple structure and easy engineering. Benhar I successfully investigated the potential of phages as targeted drug delivery carriers by using them as anti-tumor nanomaterials.[16] For chemical drug delivery, filamentous phages can load a large number of chemical drugs and have superior pharmacokinetic and delivery efficiency.[17] For gene delivery, foreign genes can be inserted into the phage genome by recombinant DNA technology or loaded by simulated phage nanoparticles by chemical or physical means.[18] Phage display has received increasing attention since it was first proposed by George P. Smith. Foreign gene sequences encoding small peptides can be displayed on the surface of phage particles (Figure 1B). Phages can be modified to target or internalize peptides by phage display,[19,20] and is distinct from any other gene and drug delivery system. In addition, selective peptides could overcome barriers in drug delivery, including specific cell binding and internalization, endosomal escape, and nuclear localization.[21] As an effective nano-vector for targeted drug delivery (Figure 1C), bacteriophage were used for targeted delivery of anticancer cell drugs.[22,23]

The filamentous phages (M13, fD and f1), which are widely used in the development of phage display systems, possess single-stranded circular DNA and are harmless to humans as biological nanomaterials,[24–26] with a length of about 1 micron and a diameter of about 7 nanometers.[27,28] It is surrounded by five structural proteins (pIII, pVI, pVII, pVIII, and pIX).[29] Phage display on filamentous phage vectors has produced many libraries of large structural complexity, of the order of magnitude $\geq 10^9$ unique sequences. These libraries contain many restricted and/or random structures that provide a rich source of targeted ligands for numerous applications.[30,31] In addition, phage display in minor coat protein PIII has been used to identify cancer-specific peptides in a variety of tumor types, including lung cancer.[32] Most importantly, the filamentous phage could enter the central nervous system (CNS) without apparent toxic effects,[33,34] as a drug carrier through the blood–brain barrier (BBB), which has great research potential in the treatment of brain diseases.[35–37] In conclusion, filamentous phages have more advantages in targeted therapy, with high specificity, sensitivity, and reproducibility. They are considered reliable performers in antibody engineering, and are increasingly important in nanobiotics and cancer research.[38–41]

Mechanisms of Nanomedicine Targeting and Advantages of Filamentous Phages

The utilization of nanomedicines has greatly improved the therapeutic effectiveness of existing drugs while minimizing the side effects associated with high doses. Nanomaterials have the ability to accumulate at tumor sites due to enhanced permeation and retention effects, which occur as a result of their passive diffusion through defective blood vessels commonly found in rapidly growing tumors. Despite the significant advancements made in passive targeting, chemotherapy resistance and tumor recurrence are still observed in various cancer types.[42] Although significant progress has been made in this "passive targeting", chemotherapy resistance and tumor recurrence are still observed clinically in many cancer types. Studies have shown that specific interactions of nanomaterials with receptors expressed on cancer cells and cancer vasculature can produce actively targeted nanomaterials, and the tumor-specific effects achieved by passive targeting can be enhanced by binding of such active targeting molecules.[43,44] The need for novel ligands and novel drug navigation strategies remains a challenge for fully active tumor targeting and drug delivery.

Compared to conventional antibodies, landscape phage virions of type f8 designed on filamentous phage vector FD-TET are an ideal targeting nanomaterial because large amounts of pure phage or phage protein can be produced in large quantities and at a low cost[31,45,46] while maintaining high purity and stability during storage. Over the past decade, a number of libraries derived from cancer cell selective phage proteins and their fusion peptides have been developed for a variety of diagnostic and therapeutic applications. These libraries have been extensively studied,[47] and their

applications include targeted nanoparticles for gene delivery to specific cells, probes for diagnosis and imaging, cancer-specific liposomes or micelles containing various therapeutic payloads, cancer-specific siRNA nanoparticles, and nanorods for photothermal therapy,[48] Additionally, filamentous phage-based "self-navigating nanomedicine" has also been "investigated".[49]

Filamentous Phage Vectors: Tumor-Targeting Mechanisms and Peptide Screening

Traditional chemotherapeutic drugs have poor accuracy on tumor cells and are prone to adverse reactions. Therefore, targeted therapy is particularly important for cancer treatment.[50–52] Peptides that specifically bind to tumor tissue can act as vehicles to guide drugs directly to the tumor, thereby greatly enhancing the precision of drug targeting.[53–55] Although monoclonal antibodies have been successfully used as anti-tumor carriers, the high molecular weight of antibodies may reduce the efficiency,[56,57] and phage peptide library has the advantage of screening small-molecule peptides, which can make up for the deficiency of antibodies, and is widely used in cancer diagnosis and treatment.[30] The use of phage-display peptide libraries to obtain tumor-specific peptides and their application in tumor targeting have been verified by scholars.[58–61]

Biological Panning of Tumor-Targeted Peptides via Filamentous Phage Libraries

Phage peptide library panning usually involves three to five rounds of specific biological panning of proteins, cells, or tumor tissues to screen for affinity peptides[62] (Figure 2). The tumor-targeted peptides screened by phage peptide library have high affinity and specificity for tumors, and have higher tumor permeability compared with antibody ligands.

Principles of Biopanning

Biopanning strategies are tailored to balance experimental control (in vitro) and physiological relevance (in vivo), ensuring that screened peptides function in the complex tumor microenvironment (TME): In vitro biopanning uses established tumor cell lines (eg, MCF-7 for breast cancer, PANC-1 for pancreatic cancer) to screen libraries under controlled conditions. This approach allows for high-throughput testing of peptide binding and easy optimization of selection parameters. For example, in breast cancer research, in vitro biopanning of M13 libraries against $HER2^+$ SKBR3 cells identified peptides with subnanomolar affinity for HER2, which were later validated for targeted drug delivery.[63] However, in vitro models lack the TME complexity (eg, stromal cells, extracellular matrix) that influences peptide binding in vivo, necessitating follow-up in animal models. In vivo biopanning injects phage libraries into tumor-bearing animal models to mimic the physiological TME, ensuring that selected peptides can navigate systemic circulation, avoid immune clearance, and bind to tumors in a living organism.[62,64] For example, in prostate cancer research, intravenously

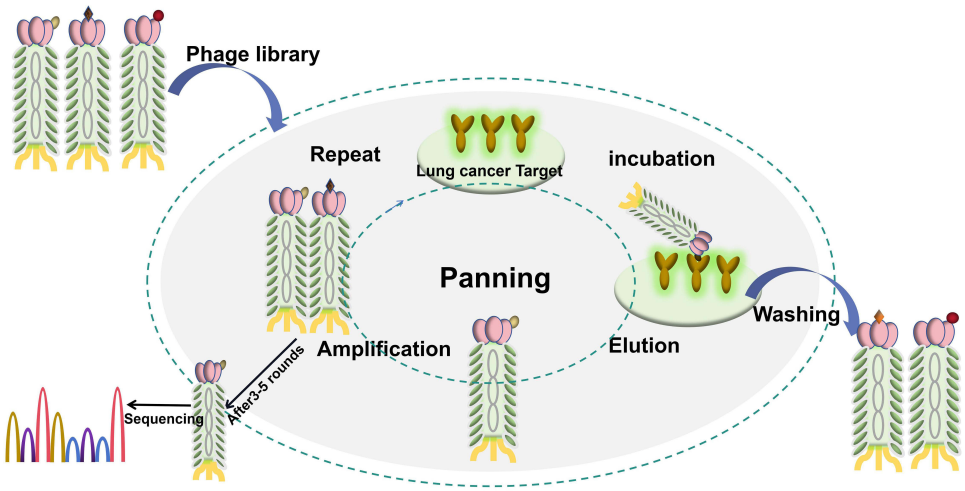

Figure 2 Screening of Lung Cancer Biomarker Specific Peptide: PIII phage library is mixed with the lung cancer target proteins. After incubation, the unbound phages are washed, and the bound phages are collected, eluted and amplified. Through 3–5 rounds in vitro screening, obtain phages specifically binding to lung cancer tumors.

injected M13 libraries were recovered from tumor tissues after 3 rounds of selection, yielding peptides that bind to prostate-specific membrane antigen (PSMA) with higher level in vivo tumor accumulation than in vitro-selected peptides.[55] This discrepancy highlights the importance of in vivo screening, as it accounts for factors like peptide stability in serum and tissue penetration—critical for translational success.

A key advantage of phage-derived peptides over monoclonal antibodies is their size and permeability: peptides (~5–20 amino acids) exhibit higher tumor permeability due to their small molecular weight, allowing them to diffuse into deep tumor tissues and bind to hidden receptors that are inaccessible to larger antibodies.[30,58,59] Additionally, phage libraries can be screened to target multiple tumor-associated antigens (TAAs) simultaneously, addressing tumor heterogeneity—a major barrier to effective cancer therapy.[61]

Functional Applications of Screened Peptides: From Targeting to Therapy

Phage-derived tumor-targeted peptides are not merely "binding molecules" but functional components of anti-tumor strategies, with applications spanning diagnosis, drug delivery, and imaging. Peptides can be conjugated to chemotherapeutics (eg, paclitaxel, doxorubicin) or toxins to enhance tumor accumulation. For instance, a prostate cancer-specific peptide (identified via M13 library screening) was linked to paclitaxel via a cleavable linker, increasing drug concentration in prostate tumors and reducing systemic toxicity (eg, neutropenia) compared to free paclitaxel.[53] This approach addresses the poor solubility and off-target toxicity of many chemotherapeutics; Peptides labeled with fluorescent dyes or radionuclides enable non-invasive tumor detection, a key step in early diagnosis and treatment monitoring. A phage-derived peptide targeting annexin A2 (overexpressed in pancreatic cancer) was used to image pancreatic cancer xenografts via fluorescence microscopy.[21] This imaging capability can also be used to guide surgical resection or monitor response to therapy. Collectively, these applications demonstrate that phage display libraries are a versatile tool for identifying functional ligands that address critical gaps in cancer therapy—from targeting to diagnosis.

Filamentous Phages as Tumor Drug Carriers: General Advantages

Beyond peptide screening, intact filamentous phages function as nanocarriers for diverse therapeutic payloads. Compared with contemporary competing nanotherapeutic platforms, Oncolytic viruses offer strong oncolytic activity but face high immunogenicity and limited payload flexibility;[65] exosomes excel at biocompatibility and BBB penetration but suffer from low yield and inconsistent targeting.[66] Lipid nanoparticles are widely used for gene delivery (eg, siRNA) but lack tumor specificity and are prone to serum clearance.[67] In contrast, Filamentous phages combine low immunogenicity (superior to oncolytic viruses), high targeting precision via phage display (surpassing exosomes and LNPs), and inherent BBB penetration (a gap for most LNPs), while supporting multi-payload delivery—addressing key limitations of these competing tools, though they currently lag behind LNPs in clinical translation maturity.[68,69]

Multimodal Payload Loading: Adapting to Diverse Therapeutic Needs

Filamentous phages support loading of multiple drug types via complementary strategies, making them applicable to a wide range of cancer therapies:

Chemical Drugs

Hydrophobic chemotherapeutics (eg, paclitaxel) often suffer from poor solubility and low bioavailability, limiting their efficacy. Filamentous phages address this by using amphiphilic phage proteins (eg, pVIII) to form micelles that encapsulate hydrophobic drugs. Wang Tao et al used PANC-1 cancer cell-specific phage proteins to prepare targeted micelles for paclitaxel delivery, improving the drug's solubility and reducing systemic toxicity (eg, weight loss) in pancreatic cancer models.[70] This approach leverages the phage's natural amphiphilicity to solve a common pharmaceutical problem.

Gene Therapeutics

Foreign genes (eg, siRNA, cytokines, suicide genes) hold promise for cancer therapy but face challenges of instability in serum and poor cell penetration. Filamentous phages protect gene payloads via two mechanisms: (1) genetic insertion into the phage genome, which shields DNA/RNA from nucleases;[18,71] (2) complexation with phage-mimetic nanoparticles, which provides additional protection and enables targeted delivery. Bedi et al developed a phage fusion protein-

liposome system for siRNA delivery: phage proteins (targeting breast cancer cells) guided liposomes to tumor cells, protecting siRNA from serum nuclease degradation.[18,72] This system demonstrates how phages can enhance the efficacy of gene therapies by combining targeting and protection.

Immunomodulators

In the era of immunotherapy, phages can be engineered to display immunostimulatory peptides or antibodies to activate anti-tumor immunity. A recombinant fd phage displaying a TNF-α fusion protein was used to treat chondrosarcoma, inducing tumor cell apoptosis and increasing $CD8^+$ T cell infiltration.[54] This application highlights the potential of phages to bridge targeted delivery and immunotherapy—a combination that is transforming cancer treatment.

Enhanced Tumor Targeting and Retention

Filamentous phages leverage both active and passive targeting to accumulate in tumors, a dual mechanism that reduces drug loss to healthy tissues and improves therapeutic efficacy. Phage-displayed peptides bind to receptors overexpressed on tumor cells (eg, EGFR, HER2), ensuring selective homing. For example, fd phages displaying a HER2-targeting peptide accumulated in $HER2^+$ breast cancer xenografts at higher levels than non-targeted phages, with minimal uptake in normal organs.[63] This specificity is critical for reducing off-target toxicity and maximizing drug concentration at the tumor site. Moreover, the phage's rod-like structure enhances retention in tumors via the enhanced permeation and retention (EPR) effect—a phenomenon where nanomaterials accumulate in rapidly growing tumors due to defective blood vessel walls and impaired lymphatic drainage.[39,42] Unlike spherical nanoparticles, the phage's high aspect ratio increases its residence time in the tumor interstitium, further enhancing accumulation.[73] This dual targeting mechanism ensures that phages not only reach the tumor but also remain there, increasing the duration of therapeutic action.

Filamentous Phages in NSCLC Therapy: Specific Applications

NSCLC accounts for ~85% of lung cancer cases, and its clinical management is plagued by interconnected challenges: high tumor heterogeneity that renders single-target therapies ineffective, a 24–44% incidence of brain metastases in advanced patients, and an immunosuppressive tumor microenvironment (TME) that limits response to immune checkpoint inhibitors (ICIs) to just 20–30% of patients.[5,74–76] Filamentous phages—with their unique capacity for targeted peptide screening, multi-payload delivery, BBB penetration, and immunomodulation—address these unmet needs as a cohesive therapeutic platform, rather than isolated tools. Below, we integrate their specific applications in NSCLC, emphasizing how each function synergizes to overcome the disease's most intractable barriers.

NSCLC-Specific Peptide Screening: Overcoming Tumor Heterogeneity

Tumor heterogeneity—manifested as variable expression of cell-surface receptors across subtypes (adenocarcinoma, squamous cell carcinoma) and even within individual tumors—is a primary reason for the failure of conventional "one-size-fits-all" therapies.[32] Filamentous phage display libraries solve this by enabling the discovery of peptides that bind to subtype-specific or patient-specific tumor-associated antigens (TAAs), laying the groundwork for personalized targeting.

In vitro screening has been pivotal for identifying peptides that match NSCLC's molecular diversity. Brown et al used three M13 phage peptide libraries to perform biopanning on 40 NSCLC cell lines representing all major subtypes, isolating 11 unique peptides with distinct affinity profiles: for example, one peptide bound preferentially to EGFR-mutant H1975 cells but showed minimal binding to EGFR-wildtype A549 cells, while another targeted squamous cell carcinoma-specific markers (Table 1).[32] This subtype selectivity is critical because it allows drugs to be delivered only to the cells most likely to respond, reducing unnecessary toxicity to healthy lung tissue. In vivo screening further refines this specificity by accounting for the TME—including stromal cells, extracellular matrix, and systemic clearance—that in vitro models omit. Zuo et al, for instance, injected a phage display loop heptapeptide library into nude mice bearing NCI-H1299 xenografts, performing three rounds of selection to enrich for phages that accumulate in tumors.[77] The resulting peptide, NSP1, bound to NCI-H1299 cells with high specificity and, when labeled with fluorescein isothiocyanate (FITC), enabled clear visualization of tumors with minimal uptake in normal lung or liver tissue—validating its utility in guiding targeted therapies.

Table 1 NSCLC-Specific Targeting Peptides Screened via Phage Display Technology and Their Applications

Targeting Peptide Name	Screening Method	Target Object/ Cell Line	Core Function	Experimental Results	References
NSP1	In vivo screening (NCI-H1299 tumor-bearing nude mice)	NCI-H1299 cells (NSCLC)	Specifically binds to tumor cells; acts as an optical molecular probe (FITC-labeled)	NSP1 does not affect cell proliferation or migration, and exhibits specific binding to NCI-H1299 cells.	[77]
ANGRPSMT/ VNGRAEAP	In vitro screening (F8/8, F8/9 peptide libraries)	CALU-3 cells (lung adenocarcinoma)	Selectively binds to lung epithelial cancer cells; guides subcellular drug localization	ANGRPSMT and VNGRAEAP are selective for the Calu-3 cell in comparison to phenotypically normal lung epithelial cells and distribute into unique subcellular fractions.	[48]
HSP1/HSP2/ HSP4	In vitro screening (phage peptide libraries)	Multiple NSCLC cell lines	Enhances binding between drug carriers and tumor cells; improves chemotherapeutic efficacy	Doxorubicin-loaded liposomes conjugated with HSPs showed significantly higher tumor inhibition rate in NSCLC animal models than non-targeted groups	[78]
11 Subtype-Specific Peptides	In vitro screening (3 peptide libraries + 40 NSCLC cell lines)	Multiple NSCLC cell lines	Matches NSCLC molecular heterogeneity; distinguishes tumor cells of different subtypes	The peptides show distinct binding profiles across 40 NSCLC cell lines and do not bind normal bronchial epithelial cell lines., which can target to tumor tissues in vivo.	[32]

Notably, patient-derived screening takes this personalization a step further. Shukla et al administered M13 phage libraries intravenously to cancer patients, then isolated tumor-homing phages from biopsy samples.[79] The peptides displayed on these phages bound exclusively to the tumor and were used to generate soluble scFv antibodies that recognized unique TAAs. This approach addresses a key limitation of preclinical models: it ensures that the targeted antigens are clinically relevant, rather than artifacts of cell culture or animal xenografts. Collectively, these screening strategies demonstrate that filamentous phages are not just tools for peptide discovery, but enablers of precision oncology —allowing therapies to be tailored to the unique molecular profile of each NSCLC patient.

Filamentous Phages as Targeted Carriers: Enhancing Drug Efficacy and Reducing Toxicity

Once NSCLC-specific peptides are identified, filamentous phages serve as ideal carriers for delivering chemotherapeutics, gene drugs, and other payloads—addressing two critical limitations of conventional NSCLC therapy: poor drug solubility (eg, paclitaxel) and off-target toxicity (eg, doxorubicin-induced cardiotoxicity). Their utility stems from their ability to combine peptide-mediated active targeting with the enhanced permeation and retention (EPR) effect—ensuring that drugs accumulate in tumors at concentrations high enough to induce cell death, while sparing healthy tissue.

For chemotherapeutic delivery, phage-based carriers have been particularly effective at improving the pharmacokinetics of poorly soluble drugs. Han-Chung Wu et al conjugated phage-derived peptides (HSP1, HSP2, HSP4) to liposomal doxorubicin, leveraging the peptides' ability to bind NSCLC cells and the liposomes' capacity to protect doxorubicin from degradation.[78] In NSCLC animal models, the targeted liposomes achieved higher tumor accumulation than non-targeted controls. In Kiattawee Choowongkomon's study, they developed the VH/VH H displayed-phage clones bound to recombinant EGFR-TK. These phages were linked molecularly to nonaarginine, which were cytotoxic to A549 and more effective than small molecular TKIs. Computerized homology modeling and intermolecular docking revealed

these phages might disrupt EGFR dimerization leading to inhibition of intracellular signaling. The humanized-cell penetrable phage nanobodies have a high potential for developing further towards a clinical application.[80]

Gene therapy for NSCLC—long limited by serum instability of nucleic acids and poor cell penetration—also benefits from phage carriers. Bedi et al. Developed a phage-liposome system to breast cancer cells, using a phage fusion protein targeting EGFR to guide liposomes loaded with siRNA against BCL-2.[72] The phage protein ensured selective binding to cancer cells, while the liposome protected the siRNA from serum nucleases and increase cancer cell apoptosis. Most patients suffering from NSCLC have EGFR overexpression. This approach offers the potential for development of new anti-NSCLC phage-siRNA-based targeted gene nanomedicines. These highlight a key advantage of phage carriers: they are not limited to a single payload type, but can deliver chemotherapeutics, gene drugs, and immunomodulators—making them versatile tools for combination therapy, which is increasingly recognized as essential for NSCLC treatment.

Phage-Mediated Immunotherapy: Reversing the Immunosuppressive TME

Filamentous phages address this by acting as both immunostimulants and targeted delivery vehicles, enhancing anti-tumor immunity while avoiding the systemic toxicity of non-targeted immunotherapies. Rasaee et al engineered M13 phages to display an ICR-62-binding peptide that mimics EGFR—a TAA overexpressed in ~30–50% of Asian NSCLC patients.[81] When used as a vaccine, these phages induced high titers of anti-EGFR antibodies and activated EGFR-specific $CD8^+$ T cells, which recognize and kill $EGFR^+$ NSCLC cells. In $EGFR^+$ NSCLC xenografts, the vaccine reduced tumor growth and prolonged median survival, demonstrating its ability to target both the humoral and cellular arms of the immune system. Li et al constructed a Trop2-specific phage display nanobody library and screened Trop2-specific nanobody. Based on this nanobody, Trop2 CAR-T cells were successfully constructed, which could kill Trop2-positive NCI-H292 NSCLC cell and increase the secretion of cytokines (IL-2, IFN-γ, and TNF-α), further validating their antitumor activity.[82] Similar,yuan et at screened and constructed of anti-Nrp-1 IgG antibody from a constructed scFv phage library. This anti-Nrp-1 IgG antibody partially restored the killing function of exhausted CD8+ T cells in malignant pleural fluid in vitro.[83] Co-culture of PBMC with A549 and the addition of anti-Nrp1-IgG enhanced the killing of A549 target cells, leading to an increase in late-stage apoptosis of target cells. Filamentous phages enhance anti-tumor immunity by serving as immunostimulants, targeted delivery vehicles and screening tools, effectively targeting both humoral and cellular immune responses against specific antigens.

Additional, phages can activate innate immune cells to produce pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6, fostering a microenvironment conducive to immune infiltration and tumor cell killing. Phage-activated dendritic cells can also release chemokines that recruit T cells and macrophages to tumor sites.[84] The immunogenic single-stranded DNA of M13 phage triggers inflammatory responses and helps counteract tumor-associated immunosuppression. Phages may also inhibit the activity of immunosuppressive cells like Tregs and MDSCs, further enhancing anti-tumor immunity.[85] Additionally, phage DNA is recognized by intracellular sensors such as TLR9, activating MyD88- or STING-dependent pathways and inducing cytokines, which collectively shape anti-tumor immune responses.[86]

Targeting NSCLC Brain Metastases: Overcoming the BBB

Brain metastases are the most devastating complication of advanced NSCLC, with a median survival of just 3–6 months.[74] The primary barrier to effective treatment is the BBB, which blocks >98% of small-molecule drugs and nearly 100% of macromolecules—including most EGFR-TKIs and ICIs.[87] Filamentous phages' unique ability to penetrate the BBB, combined with their targeting capability, makes them the only non-viral vector capable of delivering therapeutics directly to NSCLC brain metastases.

The first step in this application is identifying BBB-penetrating peptides, which guide phages across the tight junctions of the BBB. Wan et al screened an M13 phage library in mice to identify a "nose-to-brain homing peptide" (sequence: RVG29) that binds to nicotinic acetylcholine receptors on brain endothelial cells.[37] When displayed on M13 phages, RVG29 increased BBB penetration—enough to deliver therapeutic doses of drugs. Rahn et al built on this by identifying the T7 peptide, which binds to transferrin receptors on BBB endothelial cells—a receptor that is highly expressed and undergoes receptor-mediated endocytosis, further enhancing BBB crossing.[88]

To target brain metastases specifically, phages could be engineered to display both BBB-penetrating peptides and brain metastasis-specific ligands—ensuring that they cross the BBB and then bind to metastatic NSCLC cells, rather than normal brain tissue. For example, first-generation EGFR-TKIs (eg, gefitinib) have low BBB penetration, limiting their efficacy in EGFR-mutant NSCLC brain metastases. Researchers could engineer M13 phages to display both RVG29 (BBB-penetrating) and an EGFR-targeting peptide, loading gefitinib via hydrophobic interactions with phage coat proteins. In EGFR-mutant NSCLC brain metastasis models, this dual-targeted phage increased gefitinib concentration in brain tumors.

Similarly, T790M mutation—responsible for EGFR-TKI resistance in ~50–60% of NSCLC patients with brain metastases—can be targeted using phage-delivered siRNA.[89] Phages displaying T7 (BBB-penetrating) and a T790M-specific peptide were used to deliver siRNA against T790M to brain metastases.

The Prospect of Filamentous Phage in Therapy of NSCLC and Brain Metastases

Filamentous phages can be used in conjunction with chemotherapy, radiation therapy, or immunotherapy to enhance overall treatment efficacy. By recognizing tumor and organ homing peptides, phage nanoparticles have been shown to be useful for targeting cancer cells, antigen detection and drug delivery.[90] This approach promotes selective targeting of cancer cells through the use of phages and can be further optimized for selective targeted therapy and as a drug delivery vehicle.[91] Therefore, in theory, phage display strategy can be successfully applied to target lung cancer cells, especially brain metastases, for personalized treatment. Despite these promising advancements, challenges remain in the development of phage-based therapies for NSCLC and brain metastases. These include the need for rigorous preclinical and clinical testing to ensure safety and efficacy, as well as the potential for phage resistance to develop in some cases. Additionally, the scalability of phage production and the regulatory hurdles for approval need to be addressed.

Excellent Drug Delivery Systems and Personalized Treatment

We can obtain therapeutic antibodies specific to lung cancer patients, even those with brain metastases, through phage display screening technology. Numerous studies have utilized phage display libraries to identify tumor-specific ligands through panning established tumor cell lines in vitro or in animal models. However, patient-derived material offers the added advantage of clinical relevance. Filamentous phage libraries have been injected into patients without significant side effects, and a successful protocol for selecting phage display ligands in patients has been established. The M13 phage in the peptide display library (from which RGD4C/AAVP was derived) was administered continuously to cancer patients over several weeks without any adverse side effects.[92] Shukla et al performed toxicity profiles in cancer patients at different doses and in phage display library formats.[93] They then obtained and evaluated tumor-homing phage antibodies and derived soluble scFv antibodies against patient tumors and found that these antibodies were cancer-specific.[79] Filamentous Fphages can serve as excellent carriers for various anti-lung cancer drugs (Figures 1B and 3).[94] The Phage vector has stability both in vivo and in vitro. Another advantage of using Ffphages in NSCLC and brain metastases treatment is their tumor-targeting capability. The design of peptides and proteins can incorporate a variety of targeting and imaging agents, allowing for in vivo screening and monitoring of specific targets using whole phages as targeting and imaging agents.[73,95] By engineering phages to display ligands that bind to receptors overexpressed on cancer cells, such as the EGFR or the human epidermal growth factor receptor 2 (HER2), these phages can selectively home to tumor cells. This targeted approach minimizes damage to healthy cells, reducing the side effects commonly associated with conventional cancer treatments.

Accurate and personalized treatment based on specific marker analysis and corresponding targeted ligands is very important to prolong the survival of patients with lung cancer and brain metastases (Figure 4). The use of filamentous phages in individualized targeted therapy can be tailored to each patient's unique tumor profile. Personalized medicine involves analyzing the genetic makeup of a patient's tumor to identify specific mutations or overexpressed receptors that can be targeted by phage therapy. This approach ensures that the treatment is highly specific to the individual's cancer, increasing the likelihood of a positive response.

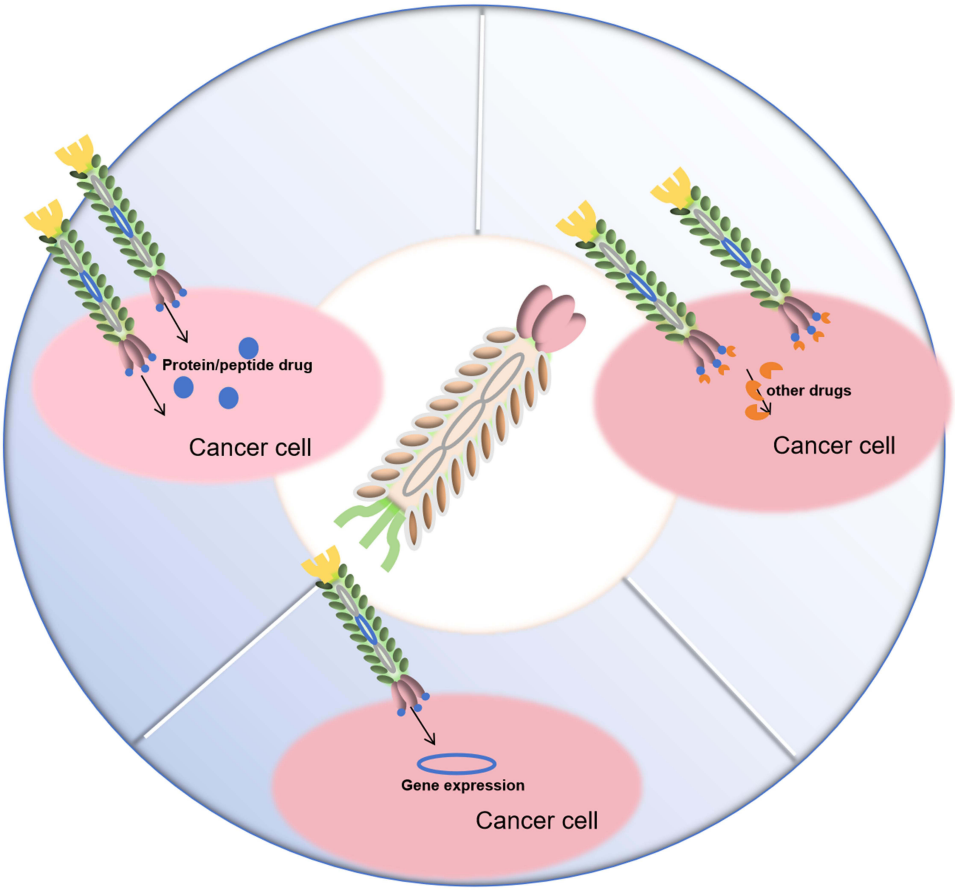

Figure 3 Phage as a drug carrier for lung cancer and its brain metastasis patients: Gene drugs and protein drugs can be directly inserted and expressed through genetic engineering; other drugs such as small molecule inhibitors, chemotherapeutic agents, and nanomedicines can be loaded through chemical modification and other methods.

Furthermore, filamentous phage does not replicate in the human body and has minimal toxic and side effects. Previous studies have successfully identified corresponding ligands by injecting a filamentous phages peptide library into patients. It is important to note that the utilization of phage nanotechnology for in vivo screening of tumor-specific ligands in lung cancer patients or using transplanted lung cancer animal models can contribute to the advancement of personalized precision diagnosis and treatment. Rod-like nanoparticles have increased affinity and selectivity for endothelial cells and increased specificity and vascular targeting to brain endothelial cells.[96] Increasing the aspect ratio of filamentous phages results in more efficient cell attachment and subsequent membrane penetration. Phages retain the bioactivity of peptides displayed on phage vectors, and these properties make filamentous phages suitable for use as vectors for the treatment of central nervous diseases.[97]

Versatile Delivery Platforms for Gene Therapy

Filamentous phage genome can be reprogrammed into a plasmid vector to carry payloads like suicide genes, tumor suppressor genes, immunomodulators, or shRNA/siRNA into tumor cells. They could deliver genes encoding cytokines (eg, IL-12, IFN-γ,TNF-α) or immune checkpoint inhibitors (eg, anti-PD-1 scFv) to mediate cancer therapy. More over, they could serve as targeted carriers for CRISPR-Cas components to edit tumor driver genes or restore tumor suppressor gene function.

Phage-Nanomaterial Therapy

Phage-nanomaterial hybrid systems represent a promising frontier in NSCLC cancer therapy. Engineered filamentous phages (eg, M13), provide excellent tumor-targeting specificity through phage display technology. Nanomaterials (eg, liposomes, polymeric nanoparticles, gold nanoparticles, or carbon nanotubes) offer superior drug-loading capacity, controlled release kinetics, and enhanced stability in the physiological environment. Phages can be combined with

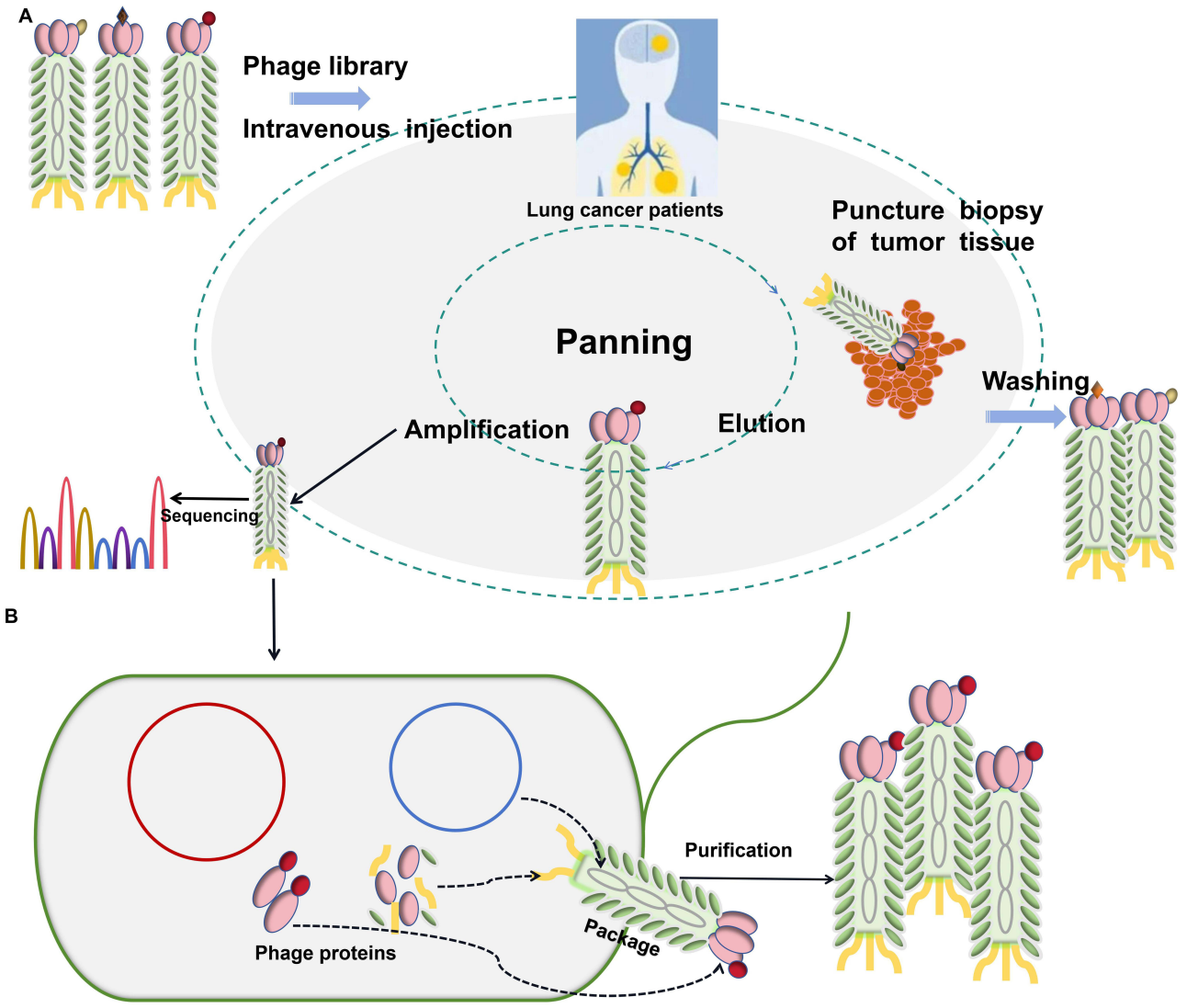

Figure 4 Phage nanotechnology-based application for personalized drug screening model in lung cancer and its brain metastasis. (**A**) After intravenous administration of a phage antibody library, upon circulation, puncture the tumor tissue to collect it, elute non-specifically bound phages, sequence and (**B**) amplify the specifically bound phages, and obtain phage drugs that can cross the blood-brain barrier.

nanomaterials to create hybrid vectors: Phages act as targeting guides, precisely delivering nanomaterial "cargo" loaded with therapeutics (chemotherapeutics, photosensitizers, or immunomodulators) to tumor sites. This significantly improves tumor accumulation while reducing off-target effects.

Clinical Translation Limitations

So far, no filamentous phage-based antitumor drugs have been approved yet. The FDA classifies engineered phages as "biological products", requiring long-term safety data (eg, 5-year follow-up of immunogenicity).[98] Although filamentous phages have low immunogenicity, few patients may produce anti-phage IgG antibodies after repeated administration, which reduces the delivery efficiency.[86] Strategies to reduce immunogenicity include PEGylation of phage surface and mutation of pVIII protein. In addition, large-scale industrial production might be difficult. Current phage production relies on *E. coli* culture, but batch-to-batch stability is poor.

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Conclusion

Filamentous phages represent a transformative platform for addressing the unmet therapeutic needs of NSCLC and its brain metastases (BM), with unique advantages in personalized targeting, BBB penetration, and multi-payload delivery. Filamentous phages enable NSCLC-specific peptide screening to overcome tumor heterogeneity, function as targeted carriers for chemotherapeutics and gene drugs to reduce off-target toxicity, modulate the immunosuppressive TME to enhance immunotherapy responses, and cross the BBB to treat otherwise refractory brain metastases.[8] These capabilities position filamentous phages as a cohesive solution to NSCLC's most intractable challenges—from therapy resistance to BBB impermeability.

However, several critical bottlenecks must be addressed to accelerate their clinical translation. First, manufacturability remains a major hurdle. Unlike lipid nanoparticles with mature industrial production processes, phages require optimized fermentation and purification protocols to reduce endotoxin levels and ensure consistent peptide display efficiency.[63] Second, immunogenicity, though lower than that of oncolytic viruses, persists: repeated administration can induce anti-phage IgG antibodies.[79] Third, NSCLC-BM-specific ligand validation is incomplete: while current ligands enable BBB penetration, few are validated for receptors overexpressed in NSCLC-BM—limiting targeting precision for brain metastases.

To surmount these barriers, near-term milestones tailored to NSCLC-BM are critical: (1) Validate 2–3 high-affinity ligands against αvβ6 and LRP1/TfR via in vivo phage display in NSCLC-BM PDX models; (2) Develop phage-guided nanocarrier hybrids (eg, Ff phage-LNP) loaded with third-generation EGFR-TKIs; (3) Establish a pilot GMP production line for phages.[99] For clinical translation, a targeted, patient-centric roadmap is critical to accelerate filamentous phage applications in NSCLC-BM.[100] First, Phase I/II trials should prioritize EGFR-mutant NSCLC-BM patients—a cohort with high unmet need. Second, Ff phages' unique BBB penetration capability (unmatched by most nanocarriers like LNPs) justifies pursuing FDA's Breakthrough Therapy Designation. In precision oncology integration, Ff phages can be paired with liquid biopsies (eg, exosomal EGFR mutation detection) to select patients likely to benefit from phage-based therapies.

Long-term, Filamentous phages may not only serve as delivery vehicles but also as "theranostic tools"—combining targeting, drug delivery, and imaging to monitor treatment response in real time.[101] While challenges remain, the preclinical success of filamentous phages in NSCLC-BM models—paired with ongoing advances in ligand engineering and manufacturing—positions them as a promising addition to the NSCLC therapeutic armamentarium, with the potential to improve survival and quality of life for patients with this devastating disease.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author Yicun Wang upon reasonable request.

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Disclosure

The authors declare no conflicts of interest.

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