Modified Bacteriophage for Tumor Detection and Targeted Therapy

Subjects: Biotechnology & Applied Microbiology

Contributor: Yuanzhao Shen , Jingyu Wang , Yuting Li , Chih-Tsung Yang , Xin Zhou

Tumor-targeted therapy is an effective method for treating tumors, and is also a focus of current tumor treatment. Due to the maturity of phage modification technology, it is now convenient, safe, and efficient to modify novel molecules on phages for targeting tumor cells or tissues. This technology has been widely used in finding new tumor-targeting molecules and in the delivery of drugs through a targeted strategy. Since the size of the phages is only nanometers long, they can cross biological barriers such as the low vascular fibrosis barrier. Due to the high surface area to volume ratio, phages can effectively carry drugs for tumor treatment compared with large molecule chemotherapeutic agents. The modified phages, as drug carriers, allow for specific targeting tumor cells while sparing normal cells. By targeting tumor cells through phage display technology, the piggybacked drug enters the cells through endocytosis and it kills or inhibits the tumor cells. It also facilitates precise treatment with less side effects, avoiding high doses of drugs and reduces tumor recurrence. Using modified phages for tumor treatment is a cost-effective and less time-consuming method.

bacteriophage (phage) genetically engineered phages phage display

1. Screening of Novel Ligands and Short Peptides as Tumor-Targeting Drugs

Currently, anti-tumor drugs are often low targeting, toxic, and prone to inducing side effects. Therefore, there is a pressing need to find high-efficient and low-toxic anti-tumor drugs. Artificial synthesized peptide drugs have the advantages of good stability, high activity, intense penetration, high affinity and specificity, and low toxicity. These features provide essential therapeutic value for tumor treatment. In contrast, many small molecular anti-tumor drugs have shortcomings of short circulation half-lives in vivo, poor water solubility, low bioavailability, unreasonable distribution in tissues, and side effects. For many years, these defects have limited the development of small molecular anti-tumor drugs. Phage gene-editing technology facilitates the discovery of potential peptide drugs by using a random phage peptide library to find the specific peptide to a target molecule. The heritability of gene-edited phages undoubtedly provides great convenience for the discovery of peptide drugs. However, considering the influence of the chemical composition and conformation, the screening of synthetic peptide drugs requires a large number of samples. In addition, it is difficult to construct an unbiased phage polypeptide library by random gene insertion. New technology is needed to assist in the construction of large capacity unbiased phage peptide libraries.

One bead-one compound (OBOC) refers to the method of synthesizing millions of random compounds so that each bead displays only one compound. It consists of three main steps: the preparation of compound libraries, the detection of library components, and the screening of the target [1]. Houghten et al. reported an efficient exponential compound synthesis method named split synthesis, which can be used to prepare combinatorial compound libraries on solid-phase supports [2]. This method divides the composite mixture into equal parts in advance. When the number of synthetic times is n for each reaction, if the type of compounds is k, the number of components in the composition library (N) is $N = n^k$. Based on a split synthesis method for solid support [3], only one chemical entity can be displayed on each solid-phase support [4]. An OBOC library consisting of millions of vectors was screened for quality to incorporate D-amino acids, unnatural amino acids, and many other organic building blocks and secondary structures into the design of phage libraries. This method has been successfully utilized to discover peptides targeting tumor vascular endothelial cells or tumor cells [5]. In 2002, Liu et al. successfully applied the OBOC approach to discover ligands for unique cell surface receptors of prostate tumor, T- and B-cell lymphomas, ovarian and lung tumors [6]. Some of these peptides demonstrated a high specific imaging property in nude mice. Hao et al. used a highly pooled and encoded OBOC peptide library in conjunction with a high-strength screening approach to identify a peptide that binds the α4β1 integrin specifically (IC50 = 2 nM) and to activate lymphoma cells [7]. Tie-2 has the potential to be used as a target gene in the gene therapy of solid tumors [8]. Wu et al. provided short peptides that could specifically bind to cells that express Tie-2 using the phage peptide library. Using the 125I labelling method, it was demonstrated that GA5 is exclusively enriched in SPC-A1 tumor cells expressing Tie-2 and that the phage vector carrying the GA5 gene allows for greater transfection efficiency. When the vector containing the p53 gene and GA5 peptide was injected into the SPC-A1 tumor-bearing mice, the tumor volume was significantly reduced [9].

2. Validation of Specific Tumor-Targeting Antibodies through Phage Display Technology

Tumor antigens such as epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), and carcinoembryonic antigen (CEA) have significant diagnostic and prognostic values as tumor biomarkers. By modifying phages, a specific antibody library including antigen-binding fragments (Fab), single-chain antibodies (scFv), and other antibody fragments can be established. This technology is beneficial given its short cycle, easy operation, and affordable price. However, due to the small molecular antibodies generated from modified phage technology, it is difficult for them to stimulate the body's immune response and allows them to reach the target cells from the intercellular space efficiently. To this end, the research focus has been to use modified phage technology to efficiently screen large molecular tumor-specific antibodies for targeted tumor therapy in recent years. In 2013, Ayat et al. constructed a pair of recombinant phage antibody libraries and successfully isolated human single-chain antibodies against the specific biomarkers of HER2 and CEA to target the lymph nodes of breast tumor patients [10]. In 2014, Lin et al. isolated the anti-human trophoblast cell surface antigen 2 (Trop2) Fab antibody from breast tumor cells using phage display technology and demonstrated that the anti-Trop2 antibody effectively reduced tumor migration and inhibited tumor growth. The anti-Trop2 antibody could also promote the death of breast tumor cells [11]. In addition, Han et al. obtained gene-edited phages specific to the

total prostate-specific antigens (t-PSA) using the f8/8 landscape phage library after three rounds of biological screening. Furthermore, they also developed sandwich enzyme-linked immunosorbent assay (ELISA) and differential pulsed voltammetry (DPV) determination systems based on the phages, both of which achieved high specificity and reliability [12].

From 2014 to 2019, the U.S. Food and Drug Administration (FDA) approved three types of anti-tumor antibodies, all of which were discovered based on phage display techniques. Ramucirumab is an anti-tumor antibody that selectively binds to extracellular vascular endothelial growth factor receptor (VEGF)-2, thereby inhibiting the induction and angiogenesis of (VEGF)-2 and the proliferation and migration of endothelial cells. In 2014, Ramucirumab was approved by the FDA for the treatment of advanced gastric or gastroesophageal junction adenocarcinoma [13]. Similar to ramucirumab, necitumumab was developed using a Dyax Fab phage display library and was approved by the FDA in 2015 for use in conjunction with gemcitabine and cisplatin as a first-line treatment of metastatic non-small cell lung tumor (NSCLC) [14]. In 2017, the FDA approved avelumab, a fully human mAb, for the treatment of Merkel cell carcinoma patients over the age of 12. To sum up, it is promising to use phage display technology to find new anti-tumor antibodies.

3. Targeted Delivery Vehicle for Chemotherapy Drugs

Since the vast majority of phages are in the nanometer scale, they have the potential to penetrate biological barriers such as the capillaries [15]. The specific phages carrying drug molecules can achieve targeted tumor therapy and reduce the damage and side effects to normal cells [16]. Drug-loaded phages targeting tumor cells can enter the cell through endocytosis in which the drug inhibits or kills the tumor cell. Phage loading technology facilitates the precise treatment of tumors with less side effects, avoids drug overdose, and reduces tumor recurrence [17]. The modified phage treatment of tumors is a cost-effective and time-saving methodology. Phages serve as excellent carriers to deliver chemotherapeutic drugs to specific sites, and their targeting can be classified as passive targeting and active targeting. The nanoscale size of phages makes it challenging for them to be cleared by the endoplasmic reticulum system.

When the filamentous phage of class Ff infects the host bacteria, the capsid protein pVI is responsible for anchoring to the host cell membrane. Therefore, the pVI protein in vitro is lipophilic and can spontaneously bind to the lipid bilayer. By displaying polypeptide drugs on capsids or linking drug-carrying liposomes to specific polypeptides, the integrity of the polypeptide drugs would not be compromised [18], and the efficiency of the targeting of phages would also improve. A study by Pastorino et al. found that liposome-targeted therapy for gliomas using doxycycline liposomes can effectively inhibit tumor growth and is a valuable tool for glioma liposome-targeted therapy [19]. The type 88 phage engineering method was used in this approach as the genome of the Type 88 bacteriophage has two distinct genes that encode the pVIII protein: a wild-type g8 and a recombinant g8. In this system, wild-type g8 can be used to help maintain the integrity of the phage pVIII protein, while recombinant g8 encodes a new master epidermal protein (recombinant pVIII) fused with a functional peptide/protein. In addition, recombinant pVIII can accommodate relatively large or structurally bulky inserts that the wild-type pVIII coat proteins cannot tolerate. Using the Type 88 phage engineering method, a new gene is inserted in the non-coding

region of the phage genome to additionally express cRGD on the pVIII protein. Compared with the M13 phages modified with either linear RGD on pVIII or cRGD on pIII, cRGD on recombinant pVIII exhibited higher internalization efficiency in ligand density and conformational structure into HeLa cells [20]. By applying phage antibody libraries to disease proteomics studies, Nagano et al. developed a new drug delivery platform to validate many protein candidates [21]. Functional mutant proteins R1antTNF were generated with enhanced receptor affinity and specificity by using phage display technology to improve the in vivo stability of biologically active proteins.

Liposome encapsulated antitumor peptides, which were developed by phage display, are widely used in clinical tumor therapy. Hölig et al. reported a high-affinity cyclic RGD lipopeptide (RGD10) targeting αv-integrin and applied doxorubicin-loaded RGD10 liposomes in an in vivo C26 colon cancer mouse model. It showed an improved efficacy compared to free doxorubicin and non-targeted liposomes. RGD10 is a tripeptide sequence selected from the phage display library against endothelial cells and melanoma, and subsequently integrated into liposomes [22]. With the same principle, Wang et al. used an 8-mer landscape library f8/8 and a bio-panning protocol against MCF-7 cells to select a landscape phage protein bearing the MCF-7-specific peptide. Subsequently, a chimeric phage fusion coat protein specific toward MCF-7 cells, identified from a phage landscape library, was directly incorporated into the liposomal bilayer of doxorubicin-loaded PEGylated liposomes (Doxil) without additional conjugation with lipophilic moieties. The results show that phage-modified doxorubicin liposomes with PEGylated liposomes loaded with MCF-7 breast tumor cells demonstrated significantly increased cytotoxicity toward target cells in vitro [23]. In addition, Mudd et al. developed a binding protein-4-targeted bicyclic toxin adduct to synthesize the conjugate BT8009 for the treatment of solid tumors. The bicyclic peptide obtained by phage display can specifically binds to Nectin-4 of tumor cells [24].

References

1. Aquino, C.; Sarkar, M. One Bead-One Compound (OBOC) Peptidomimetic-Encoded Library Synthesis via Split-and-Pool Methods. Methods Mol. Biol. 2022, 2541, 105–120.
2. Houghten, R.A.; Pinilla, C.; Blondelle, S.E.; Appel, J.R.; Dooley, C.T.; Cuervo, J.H. Generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery. Nature 1991, 354, 84–86.
3. Lam, K.S.; Sroka, T.; Chen, M.L.; Zhao, Y.; Lou, Q.; Wu, J.; Zhao, Z.G. Application of “One-Bead One-Compound” Combinatorial Library Methods in Signal Transduction Research. Life Sci. 1998, 62, 1577–1583.
4. Lam, K.S.; Salmon, S.E.; Hersh, E.M.; Hruby, V.J.; Kazmierski, W.M.; Knapp, R.J. A New Type of Synthetic Peptide Library for Identifying Ligand-Binding Activity. Nature 1991, 354, 82–84.
5. Aina, O.H.; Liu, R.; Sutcliffe, J.L.; Marik, J.; Pan, C.-X.; Lam, K.S. From Combinatorial Chemistry to Tumor-Targeting Peptides. Mol. Pharm. 2007, 4, 631–651.

6. Liu, R.; Marik, J.; Lam, K.S. A Novel Peptide-Based Encoding System for “One-Bead One-Compound” Peptidomimetic and Small Molecule Combinatorial Libraries. J. Am. Chem. Soc. 2002, 124, 7678–7680.
7. Hao, D.; Ma, B.; He, C.; Liu, R.; Farmer, D.L.; Lam, K.S.; Wang, A. Surface modification of polymeric electrospun scaffolds via a potent and high-affinity integrin α4β1 ligand improved the adhesion, spreading and survival of human chorionic villus-derived mesenchymal stem cells: A new insight for fetal tissue engineering. J. Mater. Chem. B 2020, 8, 1649–1659.
8. Tanaka, S.; Sugimachi, K.; Yamashita, Y.I.; Ohga, T.; Shirabe, K.; Shimada, M.; Wands, J.R.; Sugimachi, K. Tie2 vascular endothelial receptor expression and function in hepatocellular carcinoma. Hepatology 2002, 35, 861–867.
9. Wu, X.; Li, Z.; Yao, M.; Wang, H.; Qu, S.; Chen, X.; Li, J.; Sun, Y.; Xu, Y.; Gu, J. Identification and characterization of a novel peptide ligand of Tie2 for targeting gene therapy. Acta Biochim. Biophys. Sin. 2008, 40, 217–225.
10. Ayat, H.; Burrone, O.R.; Sadghizadeh, M.; Jahanzad, E.; Rastgou, N.; Moghadasi, S.; Arbabi, M. Isolation of ScFv Antibody Fragments against HER2 and CEA Tumor Antigens from Combinatorial Antibody Libraries Derived from Tumor Patients. Biologicals 2013, 41, 345–354.
11. Lin, H.; Zhang, H.; Wang, J.; Lu, M.; Zheng, F.; Wang, C.; Tang, X.; Xu, N.; Chen, R.; Zhang, D.; et al. A Novel Human Fab Antibody for Trop2 Inhibits Breast Tumor Growth in Vitro and in Vivo. Int. J. Tumor 2014, 134, 1239–1249.
12. Han, L.; Xia, H.; Yin, L.; Petrenko, V.A.; Liu, A. Selected landscape phage probe as selective recognition interface for sensitive total prostate-specific antigen immunosensor. Biosens. Bioelectron. 2018, 106, 1–6.
13. Frenzel, A.; Schirrmann, T.; Hust, M. Phage Display-Derived Human Antibodies in Clinical Development and Therapy. MAbs 2016, 8, 1177–1194.
14. Kumar, R.; Parray, H.A.; Shrivastava, T.; Sinha, S.; Luthra, K. Phage Display Antibody Libraries: A Robust Approach for Generation of Recombinant Human Monoclonal Antibodies. Int. J. Biol. Macromol. 2019, 135, 907–918.
15. Luqmani, Y.A. Mechanisms of Drug Resistance in Tumor Chemotherapy. Med. Princ. Pract. 2005, 14, 35–48.
16. Hamad, I.; Moghimi, S.M. Critical Issues in Site-Specific Targeting of Solid Tumours: The Carrier, the Tumour Barriers and the Bioavailable Drug. Expert Opin. Drug Deliv. 2008, 5, 205–219.
17. Manivannan, A.C.; Dhandapani, R.; Velmurugan, P.; Thangavelu, S.; Paramasivam, R.; Ragunathan, L.; Saravanan, M. Phage in Tumor Treatment—Biology of Therapeutic Phage and Screening of Tumor Targeting Peptide. Expert Opin. Drug. Deliv. 2022, 19, 873–882.

18. Petrenko, V.A.; Jayanna, P.K. Phage Protein-Targeted Tumor Nanomedicines. FEBS Lett. 2014, 588, 341–349.
19. Loi, M.; Di Paolo, D.; Soster, M.; Brignole, C.; Bartolini, A.; Emionite, L.; Sun, J.; Becherini, P.; Curnis, F.; Petretto, A.; et al. Novel Phage Display-Derived Neuroblastoma-Targeting Peptides Potentiate the Effect of Drug Nanocarriers in Preclinical Settings. J. Control Release 2013, 170, 233–241.
20. Choi, D.S.; Jin, H.-E.; Yoo, S.Y.; Lee, S.-W. Cyclic RGD Peptide Incorporation on Phage Major Coat Proteins for Improved Internalization by HeLa Cells. Bioconjug. Chem. 2014, 25, 216–223.
21. Nagano, K.; Tsutsumi, Y. Development of Novel Drug Delivery Systems Using Phage Display Technology for Clinical Application of Protein Drugs. Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. 2016, 92, 156–166.
22. Hölig, P.; Bach, M.; Völkel, T.; Nahde, T.; Hoffmann, S.; Müller, R.; Kontermann, R.E. Novel RGD Lipopeptides for the Targeting of Liposomes to Integrin-Expressing Endothelial and Melanoma Cells. Protein. Eng. Des. Sel. 2004, 17, 433–441.
23. Wang, T.; D'Souza, G.G.M.; Bedi, D.; Fagbohun, O.A.; Potturi, L.P.; Papahadjopoulos-Sternberg, B.; Petrenko, V.A.; Torchilin, V.P. Enhanced Binding and Killing of Target Tumor Cells by Drug-Loaded Liposomes Modified with Tumor-Specific Phage Fusion Coat Protein. Nanomedicine 2010, 5, 563–574.
24. Mudd, G.E.; Scott, H.; Chen, L.; van Rietschoten, K.; Ivanova-Berndt, G.; Dzionek, K.; Brown, A.; Watcham, S.; White, L.; Park, P.U.; et al. Discovery of BT8009: A Nectin-4 Targeting Bicycle Toxin Conjugate for the Treatment of Tumor. J. Med. Chem. 2022, 65, 14337–14347.