Optimization of the method for screening peptides binding to cell adhesion molecule by phage display

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Abstract

This study optimized the method for screening the phage peptide library by phage display and established an optimal screening method. The phage peptide library was screened 5 rounds using synthetic cell adhesion molecule (CAM) peptide. The amount of CAM peptide used in the first round of screening was 100 μg/mL, and the amount of CAM peptide in the second and third rounds was 50 μg/mL, the amount of CAM peptide in the 4th and 5th rounds was 25 μg/mL. After 5 rounds of screening, polyclonal phage ELISA was performed to examine the enrichment of the bound peptides by testing the binding to the CAM peptide by the phage library derived from each round of screening the original phage library. To identify the peptides that could specifically bind to the CAM peptide, the individual phage clones were randomly picked from the fifth round of phage library screening and tested by monoclonal phage ELISA for their binding to the CAM peptide. Four phage clones specifically binding to the CAM peptide were identified. This study optimized the method for screening phage peptide library and provided the optimal method for screening any peptide libraries for many different applications.

Keywords

Peptide library, phage display, ELISA.

1. Introduction

Phage display is a tool for studying protein-protein interactions and can be used to display a variety of different peptides. In 1985, George Smith first introduced and applied phage to display protein. They exposed protein fragments to the phage capsid by genetic engineering techniques to study protein-protein interactions[1,2]. Later, scientists successfully displayed small molecule antibody proteins on the surface of phage[3], enabling high-throughput screening of antibodies against specific antigens from phage libraries. The advantages of phage display in identifying binding peptides are simplicity, rapidity, efficiency, and the phage peptide library can be used many times.

The most commonly used phage display method is to fuse peptides to phage gene III capsid protein by genetic engineering techniques. The gene of displayed peptides were inserted into the plasmid expressing geneIII. The helper phage was used to infect the bacteria together to achieve the purpose of displaying the peptides on the surface of the phage[4,5]. Since most phage proteins are derived from the genome of helper phage, only a small fraction of the protein is contributed by the co-transformed geneIII plasmid, which results in the peptides being displayed on the surface of the phage in a monovalent form[6,7]. The monovalent form of the peptides in phage are more advantageous for obtaining high affinity binding peptides through screening phage library.

The phage peptide library constructed with gene III was used to screen for CAM-binding peptides in this study. By testing different experimental conditions, we found an ideal method for screening CAM-binding peptides.

2. Materials and methods

2.1 Screening phage peptide library

The CAM peptide was coated in an immunotube (Nunc) (first round: 100 μg/mL; second and third rounds: 50 μg/mL; fourth and fifth round: 25 μg/mL), overnight at 4°C. The solution in the tube was poured out and then each tube was washed three times with 4.5 mL of PBS. At room temperature, 2% BSA blocking solution was added to each tube for 2 h. The blocking solution was poured out and each tube was washed three times with PBS. Each tube was incubated at room temperature for 1 hour with 2% BSA solution containing 5×10^{12} PFU bacteriophage and then washed 10 times with PBST. Each tube was added 1 mg/mL trypsin solution and eluted the phage for 10 minutes. And then 125 μL of the phage eluate was added to 875 μL of TG1 bacteria, and placed in a preheated 37 °C water bath for 30 min. The mixed bacterial solution was diluted according to the experiment, and then 2 μL of the inoculating bacterial solution was spread to a TYE solid plate containing 1% glucose and 0.1% ampicillin. The remaining bacterial solution was all spread on another piece of TYE solid plate and incubate at 37 ℃ overnight. The 2 × TY (containing 15% glycerol) was added to the total bacterial plate, all bacteria were scraped off and collected. 50 μL of the collected bacterial solution was inoculated into 50 mL of 2 × TY liquid medium (containing 1% glucose, 0.1% ampicillin) , and cultured at 37 ℃, 230 rpm on a incubator for about 2 h until the bacterial concentration reached OD600=0.5. Two hours later, the helper phage was added to bacterial solution and placed in 37 ℃ water bath for 30 minutes in a centrifuge tube. The centrifuge tube was centrifuged at 3000 g for 15 min and the cell pellet was resuspended in 2 × TY liquid medium (containing 0.1% glucose, 0.1% ampicillin), and cultured at 25 ℃, 230 rpm for 20 h. The centrifuge tube was centrifuged at 3000 g for 20 min and added the supernatant into a sterile 50 mL centrifuge tube. Add 10 mL of 20% PEG/NaCl to the centrifuge tube, mix upside down, and ice bath for 4 h. After ice bath, the centrifuge tube was centrifuged at 4000 g for 30 min at 4 ℃, and the supernatant was discard. The pellet was resuspended in 1 mL PBS, transferred to a 1.5 mL centrifuge tube, centrifuged again at 4 ℃, 10000 g for 10 min, and the supernatant was collected and stored at 4 ℃. The saved supernatant was used for the next round of phage screening.

The above steps were repeated for 5 rounds for enrichment screening, and sequentially screened from the phage obtained in the previous round.

2.2 Polyclonal phage ELISA

NUNC 96-well microtiter plate wells was coated with 2 μg/mL CAM-PBS buffer, incubated at 37 ℃ for 2 hours. PBS was used as a control, the seven unrelated peptides(VEGF, Her2, CampH, FGF21, CD44V6, CXCR4, EGFR) were also used as controls. The plate was blocked with 2% BSA blocking solution for 2 hours at 37 ℃. After blocking, each round of the screened phage libraries were added to the plate and incubated for 60 minutes at room temperature. After incubation, each well was incubated with anti-M13-HRP antibody for 60 minutes at room temperature. TMB substrate was added to each well and incubated for 5 minutes in a dark room. The reaction was stopped with 50 μL of 1 M dilute sulfuric acid. The spectrometer measures the absorbance at OD 450 nm.

2.3 Monoclonal phage ELISA

The phage obtained by the fifth round screening was infected TG1 bacteria. The infected TG1 bacteria were spread onto a TYE plate. Thirty two monoclonal colonies were picked from the plates and inoculated into 96-well plates containing 2×TY medium (100 μg/mL ampicillin and 1% glucose) and shaken overnight at 37 ℃. The bacterial solution was aspirated from each well of a 96-well plate and inoculated into a new 96-well plate containing fresh medium, and cultured at 37 ℃, 250 rpm for 2 hours. 30% glycerol was added to the old 96-well plate wells and stored the plate at -80 degrees. The culture medium in the new 96-well plate was transferred to 1.5 mL tubes and 50 μL of KM13 helper phage was added into the tubes and water bath at 37 ℃ for 30 minutes. The infected bacteria were centrifuged at 3200 g for 10 minutes, the supernatant was discarded, and the pellet was gently resuspended in 200 μL of 2×TY medium. The bacterial cells of each tube were added to a well of a new 96-well plate and incubated at 25 ℃ for 20 h on a 250 rpm shaker. The culture in the 96-well

plate was transferred to a 1.5 mL tube. Add 20% PEG/NaCl to the centrifuge tube, mix upside down, and ice bath for 4 h. After ice bath, the centrifuge tube was centrifuged at 3200 g for 10 min to obtain the phage peptide precipitate which was used for the primary antibody in the monoclonal phage ELISA. The monoclonal phage ELISA step is identical to the polyclonal step.

3. Result and discussion

In order to obtain the peptides that could specifically bind to the CAM peptide, the phage peptide library was screened. The immunotubes were coated with the synthetic CAM peptide and subjected to five rounds library screening. The number of phage peptides added per round was 5×10^{12}. The CAM peptide was gradually reduced in the screening process, so that the peptide specifically bind to the CAM peptide could be significantly enriched. After each round of screening, the titer of the eluted phage library (P) was determined by gradient dilution, and compared with the titer of the PBS control (N). The P/N value could reflect the enrichment of the binding peptides during the screening process. The titer and P/N value after each round screening were shown in Table 1. The result of 5 rounds screening showed that the phage peptide molecules capable of binding to the CAM peptide were gradually enriched, and the P/N value increased from 3.17 in the first round to 47.8 in the fifth round. The recovery rate of phage peptide in the fifth round was significantly higher than that in the first round.

Table 1. Enrichment of the phage peptide library in each round of screening experiments

Round	Antigen（ug/mL）	Input phage（pfu）	Output phage（pfu）（P）	Output phage of negative control（pfu）（N）	Recovery Rate	P/N
1	100	5×1012	5.02×106	1.58×106	1.00×10-6	3.17
2	50	5×1012	1.46×108	1.95×107	2.92×10-5	7.49
3	50	5×1012	3.95×109	1.56×108	6.34×10-4	25.3
4	25	5×1012	1.89×1010	5.12×108	3.88×10-3	36.9
5	25	5×1012	2.81×1010	5.87×108	5.62×10-3	47.8

In order to detect the enrichment of CAM-binding peptides in the phage peptide library after screening experiments, the phage peptide libraries obtained by amplification and purification after each round of screening were incubated with the CAM peptide and tested by polyclonal phage ELISA. The result was shown in Fig. 1. As the screening process progressed, the interaction between the phage peptide molecules in the libraries and the CAM peptide became stronger, which indicates that the phage peptide molecules capable of binding to the CAM peptide were gradually enriched in the screening process.

After 5 rounds screening enrichment, we randomly picked 32 bacterial colony from the 5th round output plate. The monoclonal TG1 bacterias were cultured at a low temperature to amplify the monoclonal phage and then the culture supernatant containing the phage was collected. To identify the peptides that could specifically bind to the CAM peptide, the individual phage clones were tested by monoclonal phage ELISA for their binding to the CAM peptide. The result was shown in Fig. 2. FGF21 peptide was included as an unrelated antigen control, and PBS was also included as a control. The target antigen was the CAM peptide. We finally obtained four positive phage clones that could specifically bind to the CAM peptide, named aEp1A9, aEp1B2, aEp1B9, aEp1C3.

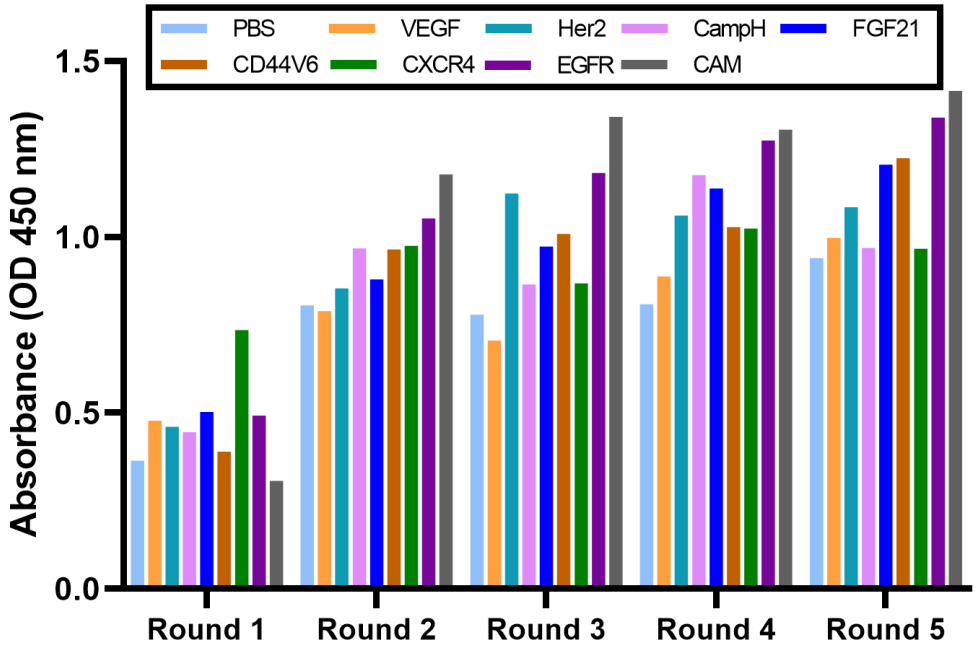

Fig. 1 Detection of screening enrichment of the phage peptide library by polyclonal phage ELISA. PBS was used as a control, the seven unrelated peptides were also used as controls, and the experimental group was tested with CAM peptide. The primary antibody was a polyclonal phage peptide obtained by screening the library, and the secondary antibody was anti-phage M13-HRP antibody.

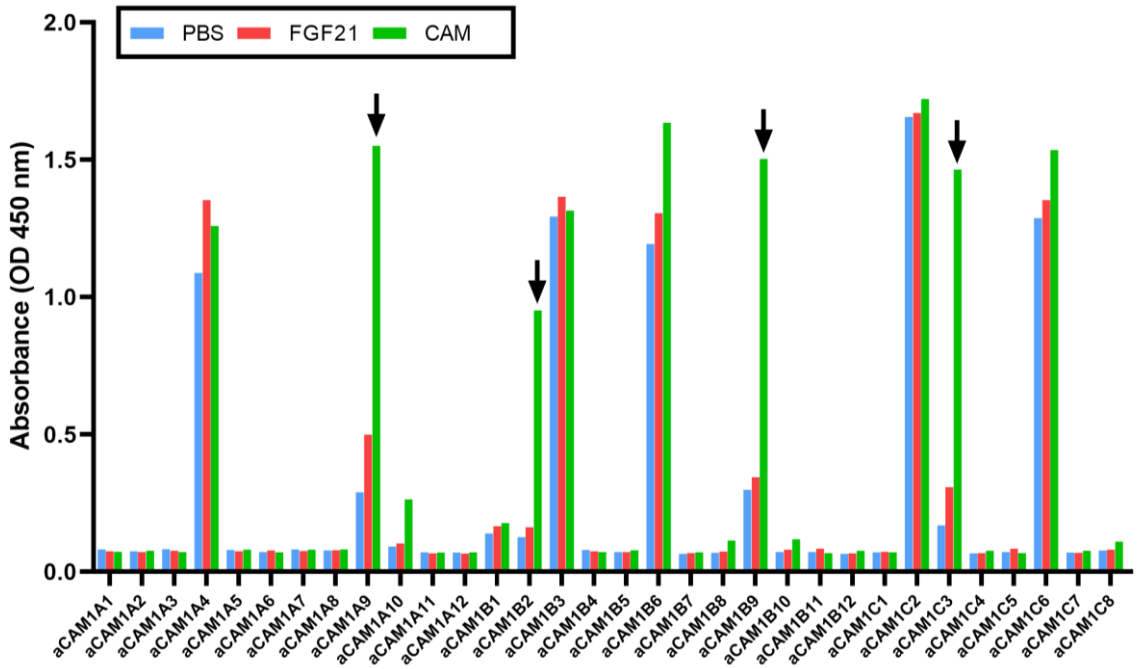

Fig. 2 Screening positive phage clones binding to the CAM peptide by monoclonal phage ELISA. The 32 phage clones were randomly picked from the phage peptide library obtained from the fifth round screening of the original phage library. FGF21 peptide was included as an unrelated antigen control, and PBS was also included as a control. The target antigen was the CAM peptide. The arrows indicate the positive phage clones.

4. Conclusion

In this study, we optimized a method for screening phage peptide libraries, and identified four positive phage clones that could specifically bind to the CAM peptide. This indicated that the method for screening phage peptide library in this study could be easily screened for binding peptides. This study optimized the method for screening phage peptide library and provided the optimal method for screening any peptide libraries for many different applications.

Acknowledgements

The authors are grateful for the financial support of the Science and Technology Program of Guangdong No. 2017A020211014 and 2013B090500109, the Science and Technology Program of Guangzhou No. 201510010123 and No. 201300000041. The authors declare no conflict of interest.

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