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Screening of the Antigen Epitopes of Basic Fibroblast Growth Factor by Phage Display

Xiang Junjian*, Zhong Zhenyu, Deng Ning*, Zhong Zhendong and Yang Hongyu
Lab of Molecular Immunology and Antibody Engineering in Life Science and Technological College, Jinan University, Guangzhou, 510632, China

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In order to investigate the epitope of basic fibroblast growth factor (bFGF) and its immunogenicity, the epitopes of bFGF were screened from the phage display library with monoclonal antibody GF22, which can neutralize the bio-activity of bFGF. By three rounds of screening, the positive phage clones with bFGF epitopes were selected, which can effectively block the bFGF to bind with GF22. Sequence analysis showed that the epitopes shared a highly conservative sequence (Leu-Pro-Pro/Leu-Gly-His-Phe/Ile-Lys). The sequence of PPGHFK was located at 22-27 of the bFGF. The specific immuno-response of mouse could be highly induced by phage clones with the epitopes. And the anti-bFGF activity induced by LPGHFK was 3 times higher than the original sequence, which showed that the mimetic peptide LPLGHIK might be used as a tumor vaccine in the prevention and treatment of tumor.

Keywords: bFGF, Epitope, Mimetic peptide, Phage display

Introduction

bFGF belongs to the fibroblast growth factor (FGF) family, which is a potent factor in normal and diseased tissues. It plays key roles in the proliferation and differentiation of cells, in the occurrance and transfer of tumor, and embryo development by binding with the high affinity receptors of cell surface (Lin, 1999). Baird (1988) found that two short peptides containing 25-69 and 24-121 amino acids of bFGF respectively could competitively bind with the bFGF receptor (bFGFR). Some shorter peptides (35~51,107~116) can also bind with the bFGFR. And some other receptor binding sites have been reported. Kurokawa (1989) demonstrated that the receptor binding site of bFGF is at the sequence of 105-115 amino acids. Ray (1997) reported that 68-77 amino acids of bFGF is the receptor binding epitope in nerve cells. Reinherz (1999) reported that the Ile at the sixth site might play key roles in the binding of major histocompatibility complex calss II (MHCII) molecule and antigen and recognizing of T cell receptor (TCR). The sequence of LPLGHIK is less conservative to the natural sequence of bFGF, but has better antigenicity than LPPGHFK. Perhaps the higher heterogeneity of LPLGHIK makes the recognition of TCR easy, or the Ile at the sixth site can increase the binding ability of the antigen epitope and MHCII molecule and TCR.

By using phage display technology, some mimetic peptides binding with bFGFR can be selected and act as a vaccine to induce anti-bFGF antibody. In this paper, the conservative amino acid sequence binding with GF22 has been selected from the random 7-mer peptide library using the phage display technology, the antigen epitopes of bFGF have been found, and the relations between the structure and function of the epitopes have been analyzed. The mimetic peptide, which can bind with bFGFR directly, has been obtained. The results showed that the mimetic peptides have good antigenicity and might be used as an anti-tumor vaccine.

Materials and Methods

Materials Anti-human bFGF monoclonal antibody (GF22) was bought from Calbiochem Co. (San Diego, USA). HRP-conjugated goat anti-mouse IgG was from Sino-American Biotech Co. A random 7-mer phage display library (with 2.8×109 complexity) was bought from New England Biolabs Co. (Beverly, USA), PEG8000 from Sino-American Biotech Co., IPTG/X-gal from Tian Xiang Ren Co., 96-well ELISA plates from Greiner. bFGF model designed by computer simulation was performed in Abmaxis Co. Primer: 5'-CCCTCATAGTTAGCGTAACG-3' was synthesized by Gene Co.

Screening of phage library The microtiter plate was coated with

*To whom correspondence should be addressed.
Tel: 86-20-85223259; Fax: 86-20-85227003
E-mail: tdengn@jnu.edu.cn; txjj@jnu.edu.cn

GF22 (10 μg/mL, 100 μL), blocked with BSA for 1 h. The phage library was diluted about 10 pfu/100 μL with TBS-T (0.1% Tween-20, pH7.4), incubated with the immobilized GF22 for 7 h at room temperature. The wells were washed with TBS-T to remove the non-binding phages, and then washed 2 times with bFGF/TBS-T (800 ng/mL, pH 7.4) and one time with glycine buffer (pH 2.2) to elute the binding phages. The elution sample was collected in tubes. The titre of the eluted phages was calculated and amplified. Two additional screenings were performed with the same method.

Dot hybridization The single phage clones were selected and amplified: 30 μL TG1 solution incubated over night was added into 3 mL LB medium, and single phage clones were picked out and added in and incubated with vibration for 5 h at 37°C. The phage solution was centrifuged at 10,000 rpm for 10 min. The supernatant was transferred to another tube, the phages were deposited with 20% PEG8000/NaCl and dissolved in 50 μL TBS.

The nitrocellulose membrane 0.25 cm^2 was dripped with 1 μL phage solution, dried at room temperature, and blocked with BSA for 1 h, incubated with 1 : 5,000 anti-bFGF IgG at 4°C for 3 h, added with 1 : 10,000 goat anti-mouse HRP-conjugated IgG and colored by O-Phenylenediamine Dihydrochloide (OPD). The wild phage vcsm13 was used as a negative control.

Sequencing Single phage clones were amplified and their DNA was extracted. The DNA sequences of positive phage clones were analyzed by Gene Co.

Competitive ELISA analysis A 96-well plate was coated with 100 ng/mL bFGF at 4°C overnight, blocked with BSA for 2 h at room temperature. 50 μL GF22 (1:10,000) and 50 μL positive phage solutions were added to each well in different concentration and incubated for 2 h at room temperature. The plate was washed with PBS-T, and 1 : 10,000 goat anti-mouse HRP-conjugated IgG was added, incubated at 37°C and colored with OPD. The absorbance value was determined at 490 nm.

Immunogenicity analysis The mice were immunized with phages containing mimetic peptides of LPPGHFK and LPLGHIK. The mice serum immunized with bFGF was the positive control and the mice serum immunized with vcsm13 (10^{13} pfu) was the negative control. The immunized serum was separated from the tail after two extra-immunizations. The anti-bFGF activities of the different serum were detected by ELISA. A 96-well plate was coated with 100 ng/mL bFGF at 4ºC overnight, blocked with BSA for 2 h at room temperature. 50 μL the different immunized sera (diluted with PBS in 1 : 20, 1 : 40, 1 : 80) were added in and incubated for 2 h at 37°C. The plate was washed with PBS-T, and the goat anti-mouse HRP-conjugated IgG (1 : 10,000 diluted with PBS) was added, incubated at 37°C and colored with OPD. The absorbance value was determined at 490 nm. The other 96-well plate was coated with vcsm13 (10^{14} pfu/ml) and the other steps were the same of above. The value of which coated with vcsm13 was treated as background.

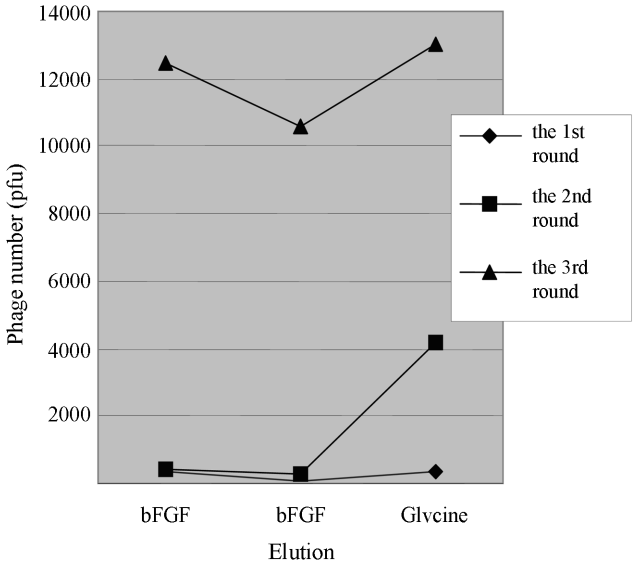

Fig. 1. Results of 3 rounds of elution.

Results

Screening of phage library The positive phage clones binding with GF22 were screened, eluted, calculated and amplified. The results of 3 rounds of elution were shown in Fig. 1. The high affinity and specific positive phage clones were obtained after three rounds of elution.

Dot hybridization The results of dot hybridization were shown in Fig. 2. A is the phage clones in the first and second screenings. 20 clones were eluted and amplified every time and 120 clones were obtained in the end. B is the phage clones after the third screening. 30 clones were eluted and amplified every time and 90 clones were finally obtained. 207 positive clones were obtained in total. The results indicated that the mimetic peptides expressed on the envelop of phages have affinity with monoclonal antibody GF22.

Sequencing The DNA sequences of 10 positive phage clones were sequenced. A conservative sequence LP(P/L)GH(F/I)K was found. And the sequence of PPGHFK is the same as that of 22-27 amino acids of bFGF, which indicated that GF22 could bind with this epitope.

Competitive ELISA analysis The result of competitive ELISA of the two different mimetic peptides LPPGHFK and LPLGHIK was shown in Fig. 3. Vcsm13 was used as a negative control. The results showed that the phage clones with peptide of LPPGHFK or LPLGHIK could competitively bind with bFGF.

Immunogenicity analysis The mice were immunized with

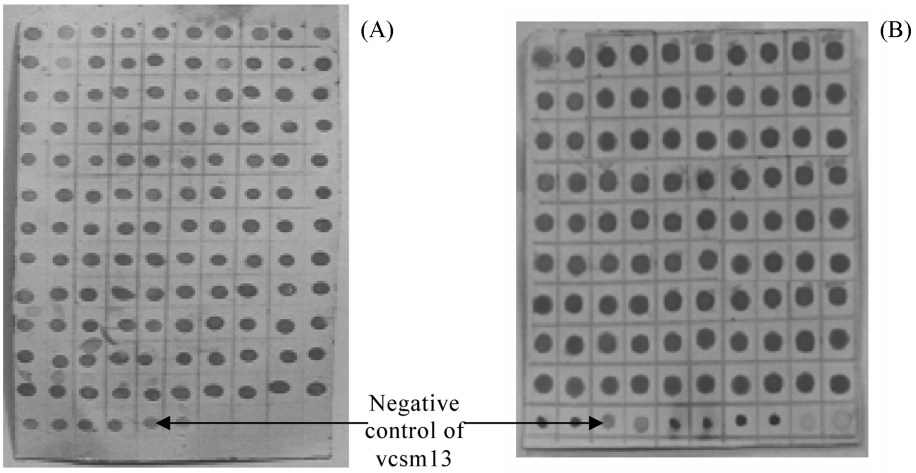

Fig. 2. Results of dot hybridization. (A) is the phage clones in the first and second screenings in which 120 clones were obtained. (B) is the phage clones after the third screenings in which 90 clones were obtained.

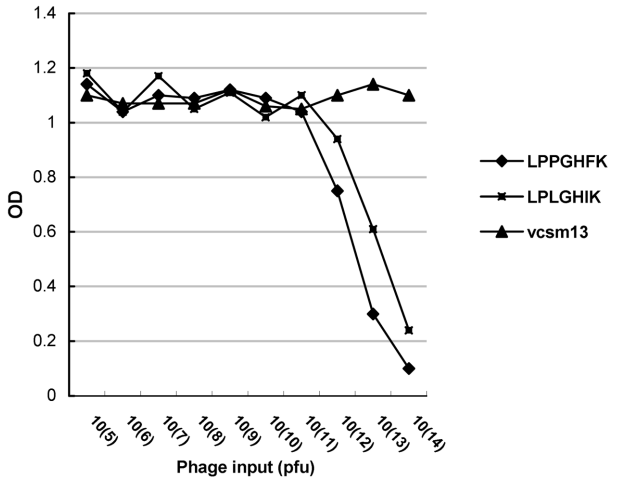

Fig. 3. The competitive ELISA analysis of the two different mimetic peptides.

the positive phage clones containing the sequence of LPPGHFK or LPLGHIK, and the positive control was immunized with bFGF. The results were shown in Fig. 4. The activity of anti-bFGF of the serum from the immunized mice with bFGF was treated as 100%. The activity of the serum immunized with LPLGHIK was equivalent to 33%, and that of the serum immunized with LPPGHFK was equivalent to 11%.

Discussion

bFGF plays key roles in tumor growth and fibrosis of lung and liver cells. It can promote the production of new blood vessels and accelerate the angiogenesis and transfer of tumor cells (Lin, 1999). The inhibition of biological activity of bFGF can effectively inhibit the growth and aggression of tumor. The epitope peptide screened by phage display can induce the body to produce anti-bFGF antibody, which can effectively inhibit the biological activity of bFGF. So the epitope mimetic peptide could be used as a tumor vaccine. Furthermore, the epitope mimetic peptide can inhibit the bio-activity of bFGF by binding with the bFGFR competitively. And the anti-bFGF antibody is most likely to bind with the domain of bFGFR too, because the bFGFR has 3 extracellular Ig-like loops regions, which are the same as the antibody.

The mimetic peptides with high affinity of bFGFR can be selected from the phage library by using anti-bFGF antibody, which is very important to analyse the interaction between the mimetic peptide and bFGFR, to diagnose, predict the occurrence and development of tumor, to study the possibility of peptide vaccine specific to inhibit tumor angiogenesis.

The epitope mimetic peptides of bFGF were screened from the random 7-mer phage display library by using anti-bFGF monoclonal antibody GF22. The amino acid sequences of the epitopes are highly conservative. The anti-bFGF activity induced by the epitope of LPLGHIK was 3 times higher than that of the epitope of LPPGHFK, which is about 33% of the immunized serum of bFGF. It was demonstrated that the epitope mimetic peptide of LPLGHIK could be used as a potential vaccine to induce the production of anti-bFGF antibody.

Compared with human bFGF, the mimetic peptides of LPLGHIK and LPPGHFK are very conservative, and the epitope of LPPGHFK is almost the same as the sequence of FPPGHFK of bFGF except one leucine replaced by phenylalanine.

The sequence of PPGHFK of bFGF is the same in human, mouse and big mouse (Cristopher, 1986). The mimetic peptide containing this sequence could induce high-level production of anti-bFGF antibody in the immunized mouse, which indicated that the sequence of PPGHFK is a key epitope of bFGF. It can be hypothesized that the antibody reduced by the epitope of PPGHFK can competitively bind

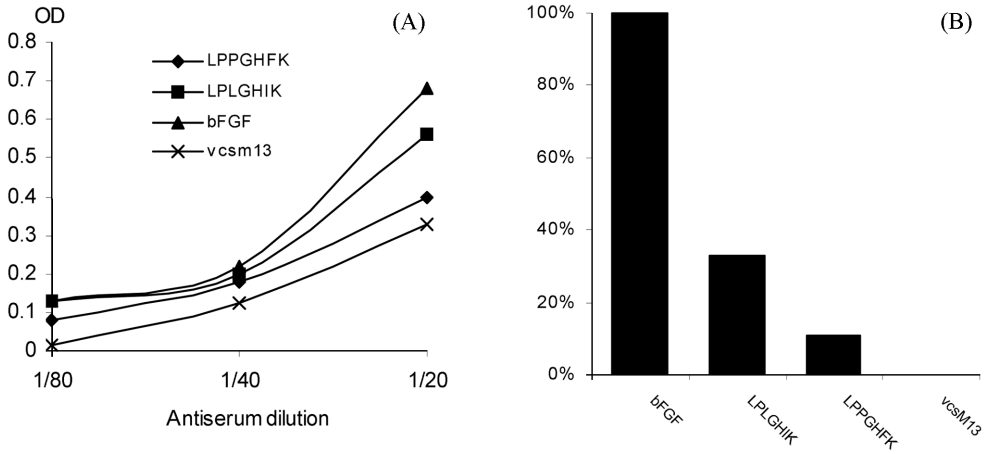

Fig. 4. Affinity activities of the serum from the mice immunized with mimetic peptides. (A) ELISA analysis of serum immunized with mimetic peptides. (B) Percentages of activities of mimetic peptides. The bFGF was used as positive control and vesm13 was used as negative control.

with the bFGFR, and inhibit the activity of bFGF.

In conclusion, the mimetic peptide of bFGF could be used as a tumor vaccine to induce the production of anti-bFGF antibody to inhibit the production of new blood vessels and the angiogenesis and transfer of tumor cells.

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