Oncology and Translational Medicine
DOI 10.1007/s10330-019-0356-6

June 2019, Vol. 5, No. 3, P119–P122

ORIGINAL ARTICLE

Identifying peptides that specifically bind to MDA-MB-468 breast cancer cells*

Xiaoli Dai (✉), Qing Zhang, Huili Zhang, Hongtao Xu

Clinical Medical College, Jiangsu Medical Vocational College, Yancheng 224005, China

Abstract

Objective To use phage display technique to screen for small polypeptides that specifically bind to MDA-MB-468 cells.

Methods A random heptapeptide phage display library was used for *in vitro* screening against target MDA-MB-468 cells. SC1180 cells were used for subtractive selection. High-affinity phage DNA was extracted, and peptides were sequenced.

Results (1) The original library capacity of the polypeptide library was 2×10^{13} pfu/mL, and phage titer was determined over 4 rounds. The average library capacity was 1.8×10^{13} pfu/mL. (2) Subtractive screening showed that the phage library volume of each round was 1.8×10^{12} pfu/mL, and that there was an enrichment effect in each subsequent round. Screening was stopped after the fourth round. (3) PCR results showed that the size of 39 products (78.0%) and 11 products (22%), were 300 bp and 258 bp, respectively. Thirty positive phages were selected for DNA extraction and sequencing, and the corresponding amino acid sequence was LMTRXSK. The sequence had no homology with known genes or proteins.

Conclusion Using the phage display technique, we identified that the short polypeptide, LMTRXSK, specifically binds MDA-MB-468 human breast cancer cells.

Received: 7 May 2019
Revised: 8 June 2019
Accepted: 17 June 2019

Key words: phage; breast cancer; specific binding peptide

Breast cancer occurs primarily in women (approximately 99% of cases) and is caused by malignant tumors in the epithelial tissue of the breast. In situ breast cancer is not fatal, but cancer cells can aberrantly alter their surrounding networks and can spread through the whole body through vascular or lymphatic channels [2–3]. Treatments can be targeted at the cellular or molecular levels, targeting known carcinogenic sites. This is advantageous in killing only cancer cells, and can avoid toxic side effects from traditional radiotherapy and chemotherapy methods [4–5]. Therefore, identification of target sites in cancer tissues has become an important topic in cancer therapy research. To explore targeted therapy for breast cancer, we used phage display technology to screen for small molecular polypeptides that specifically bind to MDA-MB-468 human breast cancer cells.

Materials and methods

Cell lines and main reagents

Human breast cancer cells (MDA-MB-468) and wild-type human breast cells (SC1180) were purchased from Kingsley Biotechnology Co. Ltd (USA). Phage display heptapeptide library kit was purchased from New England Biolabs (NEB, USA). M13 phage single-strand DNA rapid extraction kit was purchased from Booxis (Tianjin) Biotechnology Co. Ltd (China). Dulbecco's Modified Eagle Medium (DMEM), Dulbecco's phosphate buffered saline (DPBS), and HEP were purchased from Gibco-BRL (USA). PCR primers were synthesized by Shanghai Yingjun Biotechnology Co. Ltd (China).

In vitro screening of phage display

✉ Correspondence to: Xiaoli Dai. Email: houxiangcai254@163.com
* Supported by a grant from the Jiangsu Provincial Health and Family Planning Commission (No. H201640).

heptapeptide library

MDA-MB-468 and SC1180 cells were cultured in DMEM medium containing 10% fetal bovine serum (FBS) in culture dishes treated with poly-lysine. MDA-MB-468 cells were incubated with bovine serum albumin (BSA) solution for 1 h. Then, phage was added to display the heptapeptide library, and the phage was eluted with 0.2 M glycine. The eluent was moved into the cell culture dish containing SC1180 cells. After 1 h, the supernatant, which now contained the screened phage, was purified by polyethylene glycol (PEG) method, and was used in the next round of screening. In the second, third and fourth rounds, incubation time of peptide library and MDA-MB-468 cells was reduced to 40 min, 30 min, and 20 min, respectively. Adsorption time of eluent and SC1180 cells was increased to 70 min, 90 min, and 105 min, respectively.

PCR amplification of phage DNA

The volume of the screened products in the fourth round were 20 L. The metal bath was centrifuged for 3 min after 10min. The supernatant (1 L) was used as the template. The PCR mixture contained: 0.5 L dNTP (concentration 10 mmol/L), 0.25 L DNA polymerase (5 U/L), 2.5 L reaction buffer, 1 L upstream primer, 1 L downstream primers, and 1 L DNA template. PCR conditions were: (1) 94°C for 2 min, (2) 30 cycles of 56°C for 30 s, 94°C for 30 s, 68°C for 30 s, (3) 68°C for 5 min.

Sequencing of phage clones

Nucleic acids were extracted with the positive clone kit. DNA was sequenced by Shanghai Yingjun Biotechnology Co. Ltd. (China) using the 96 g primer, 5'-hoccctcatagttagcgtaacg-3'. Homology of polypeptide sequences with known protein sequences was analyzed using NCBI BLAST website.

Results

Phage polypeptide library capacity determination

The original library capacity of the polypeptide library was 2×10^{13} pfu/mL, and phage titer was determined over 4 rounds of screening. The average library capacity was 1.8×10^{13} pfu/mL, shown in Fig. 1.

Phage peptide library enrichment effect

Cancerous MDA-MD-468 cells were used as the target cells, while the wild-type SC1180 cells were used to adsorb phages for subtraction selection. Phage library in each round was maintained at 1.8×10^{12} pfu/mL, and an enrichment effect was observed in each subsequent round.

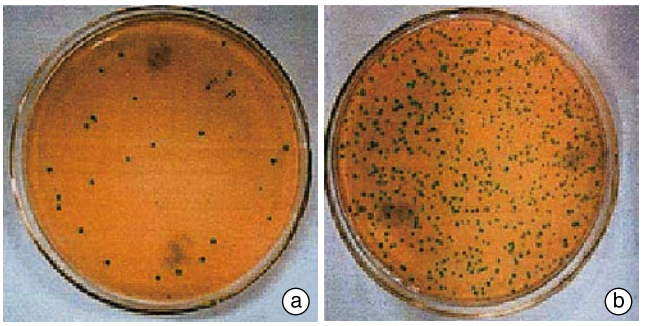

Fig. 1 Phage titer determination. (a) Phage titer determination after the first round of screening; (b) Phage titer determination after the fourth round of screening

Table 1 Recycling amount and enrichment effect of 4 rounds of selection

Times	Inputs (pfu)	Recycled (pfu)	The recovery rate	Enrichment of multiple
1	1.8×10^{12}	4.0×10^{5}	2.22×10^{-7}	–
2	1.8×10^{12}	2.5×10^{6}	1.39×10^{-6}	6.3
3	1.8×10^{12}	1.7×10^{7}	9.44×10^{-6}	6.8
4	1.8×10^{12}	2.0×10^{7}	1.11×10^{-5}	1.2

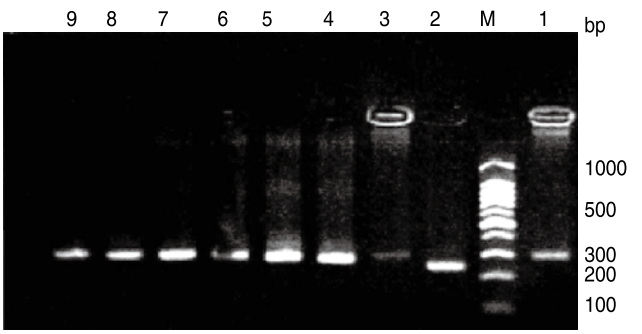

Fig. 2 PCR electrophoresis of bacteriophage DNA

By the fourth round, there was no significant difference from the third round, so the screening was suspended. Phage enrichment in breast cancer cells increased by approximately 51 times (Table 1).

PCR amplification of phage DNA

The sizes of 39 products (78.0%) and 11 products (22%), were 300 bp and 258 bp, respectively (Fig. 2).

Sequence analysis of positive phage DNA

Thirty positive phages were selected for DNA extraction and sequencing. DNA sequences were analyzed with Chromas software. The DNA sequence translated to the amino acid sequence, LMTRXSK. The sequence showed no homology with known genes and proteins (Fig. 3).

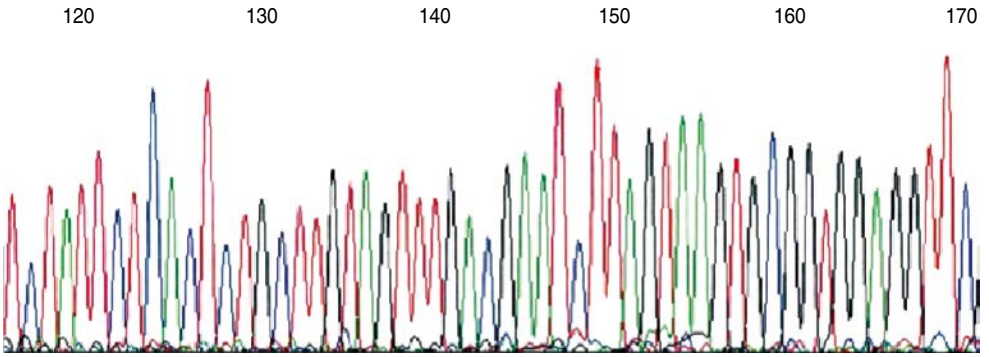

Fig. 3 Results of PCR product sequencing

Discussion

Increasing incidence of cervical spondylosis, malignant tumors, cardiovascular disease, and cerebrovascular disease in women correlates with increasing undertaking of high-pressure roles in society [6–7]. Breast cancer is caused by one of the most common types of malignant tumors in women. In some regions in the world, the incidence of breast cancer is second only to lung cancer, but the fatality rate ranks first among malignant tumors [8]. Compared to Europe and America, the incidence of breast cancer in China is higher, however, the cure rate is lower. The survival rate of patients has improved due to advancements in surgical techniques and chemotherapy regimens. However, there have been no recent breakthroughs in improving patient survival [9–10]. Therefore, more research is being focused on looking for targeted therapies with stronger specificity and fewer side effects.

Phage display is an experimental technique in which peptides are displayed on the phage surface, and the target peptides are then extracted and isolated [11]. A variety of malignant tumor-targeting peptides have been validated, and have been used in early diagnosis or targeted treatment of malignant tumors [12]. Phage display is an experimental technique in which peptides are displayed on the phage surface and the target peptides are extracted and isolated [13]. In this study, MDA-MB-468 breast cancer cells were targeted, and wild-type SC1180 cells were selected as adsorption cells to screen for specific peptides that bind human breast cancer cells. After four rounds of screening, phage enrichment in breast cancer cells increased by about 51 times, indicating that phage specifically bound to MDA-MB-468 was enriched. DNA extraction and sequencing of 30 positive phages revealed that the amino acid sequence of the peptide was LMTRXSK.

In conclusion, this study used phage display technology to screen and identify a small molecule polypeptide, LMTRXSK, that can specifically bind to MDA-MB-468 human breast cancer cells. This may be a potential candidate for breast cancer diagnosis and targeted therapy.

References

1. Leung YK, Govindarajah V, Cheong A, *et al*. Gestational high-fat diet and bisphenol A exposure heightens mammary cancer risk. Endocr-Relat Cancer, 2017, 24: 345–358.
2. Lin W, Modiano JF, Ito D. Stage-specific embryonic antigen: determining expression in canine glioblastoma, melanoma, and mammary cancer cells. J Vet Sci, 2017, 18: 101–104.
3. Roy S, Banerjee S, Chakraborty T. Vanadium quercetin complex attenuates mammary cancer by regulating the P53, Akt/mTOR pathway and downregulates cellular proliferation correlated with increased apoptotic events. Biometals, 2018, 31: 647–671.
4. Matsuda N, Lim B, Wang X, *et al*. Early clinical development of epidermal growth factor receptor targeted therapy in breast cancer. Expert Opin Investig Drugs, 2017, 26: 463–479.
5. Kiderlen M, van de Velde CJH, Liefers GJ, *et al*. Targeted therapy in older women with breast cancer – What's the target? Eur J Surg Oncol, 2017, 43: 944–948.
6. Li R, Zhang L, Yang JX, *et al*. Analysis of inpatient payments of breast cancer patients with different medical insurance coverages in China (mainland) in 2011–2015. Chin J Cancer Res, 2017, 29: 419–425.
7. Dinegde NG, Xuying L. Awareness of Breast Cancer among Female Care Givers in Tertiary Cancer Hospital, China. Asian Pac J Cancer Prev, 2017, 18: 1977–1983.
8. Zhang NQ, Ho SC, Mo XF, *et al*. Glucosinolate and isothiocyanate intakes are inversely associated with breast cancer risk: a case-control study in China. Br J Nutr, 2018, 119: 957–964.
9. Li Q, Yang Z, Fan J, *et al*. A nation-wide multicenter 10-year (1999-2008) retrospective study of chemotherapy in Chinese breast cancer patients. Oncotarget, 2017, 8: 75864–75873.
10. Wu J, Fang M, Zhou X, *et al*. Paraoxonase 1 gene polymorphisms are associated with an increased risk of breast cancer in a population of Chinese women. Oncotarget, 2017, 8: 25362–25371.
11. Sakamoto K, Kamada Y, Sameshima T, *et al*. K-Ras(G12D)-selective inhibitory peptides generated by random peptide T7 phage display technology. Biochem Biophys Res Commun, 2017, 484: 605–611.
12. Jafari B, Hamzeh-Mivehroud M, Moosavi-Movahedi AA, *et al*. Identification of novel single-domain antibodies against FGF7 using

phage display technology. SLAS Discov, 2018, 23: 193–201.
13. Arab A, Robati RY, Nicastro J, *et al*. Phage-based nanomedicines as new immune therapeutic agents for breast cancer. Curr Pharm Des, 2018, 24: 1195–1203.

DOI 10.1007/s10330-019-0356-6
Cite this article as: Dai XL, Zhang Q, Zhang HL, *et al*. Identifying peptides that specifically bind to MDA-MB-468 breast cancer cells. Oncol Transl Med, 2019, 5: 119–122.