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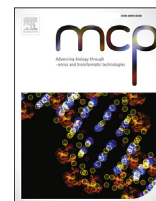

The further characterization of the peptide specifically binding to gastric cancer

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ABSTRACT

Targeting peptide has been considered to be useful as a small molecule probe leading to multifunctional properties for both imaging detection and targeting therapy. Thus, the identification of novel targets is urgently needed to develop innovative agents to effectively control gastric cancer metastasis and progression. Previously, we reported a novel 12-mer peptide, GP-5 (IHKDKNAPSLVP), binding to gastric carcinoma (GC) cells specifically and sensitively, and it was screened by using a phage displayed peptide library and primarily analyzed. In this study, it was further identified via fluorescence microscopy, flow cytometry, tissue chip and other methods. Our results indicated that the peptide GP-5 presents a particularly high affinity and specificity to GC cells and tissues, whereas only background detection occurred with other control cancer cells, cancer tissues or normal tissues. Taken together, all results support that the peptide GP-5 is a potential candidate to be developed as a useful molecule fragment for the imaging detection and targeting therapy of GC.

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1. Introduction

Gastric carcinoma (GC) ranks fourth among the most commonly diagnosed tumors and is the second leading cause of cancer-related deaths worldwide [1]. Although surgery is the effective method for GC patients, chemotherapy is the primary treatment for patients with advanced GC [2]. New methods for the early detection of GC are urgently needed. Early detection is of paramount importance to improve the 5-year survival rate of the patients. But the current white light endoscope has limited effectiveness for early GC screening, and the current chemical therapeutics' huge side effects cause the poor prognosis of their usage because of their low targeting property. Therefore, it is imperative to develop the sensitive and effective methods for the targeting detection and therapy of GC.

The tumor specifically binding peptides are recently considered as an available direction element candidate for the targeting therapy of cancer. Phage-displayed peptide library, a powerful ligand peptide discovery route, has been widely and successfully employed to uncover novel peptide with tumor specificity against in vitro purified cell surface proteins [3,4], intact cells [5] and in vivo tissue [6,7]. Promising peptides have been identified without predominant notions, which lead to the determination of corresponding cell surface receptors, such as integrin [8,9], aminopeptidases [10] and growth factor receptors [11]. These receptors can serve as valuable molecular targets in the development of targeted therapy. In our previous studies using whole-cell subtractive panning of the phage displayed 12-mer peptide library, we have identified peptides including GP-5 that bind specifically to SGC-7901 cells [12].

In the present study, to further characterize the specificity and sensitivity of GP-5 and identify it as a potential targeting diagnosis probe and drug delivery guider to develop the new methods for the GC clinical needs, we projected the characterization with immunocytochemistry and immunohistochemistry methods.

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2. Materials and methods

2.1. Cell lines and cell culture

The human GC SGC-7901 cell line and the human embryonic kidney cell line HEK293 were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco, BRL, Gaithersburg, MD, USA), supplemented with 10% (v/v) fetal bovine serum (Gibco) at 37 °C in a humidified atmosphere of 5% CO_2.

2.2. Flow cytometry assay for GP-5 specifically binding

SGC-7901 cells were plated into 12-well plates overnight, once the cells reached 90% confluence, incubated with culture medium containing 1.5% BSA at 37 °C for 30 min for blocking and then with FITC-conjugated GP-5 (5 μM) at 37 °C for 40 min. The irrelevant peptide (5 μM) was used as a negative control. After being washed three times with PBS, cells were subjected to flow cytometry (Guava Easy Cyte 8HT, EMD Millipore, USA). Data acquisition and analysis were carried out using the software, FACS Calibur cytometer, supplied with the flow cytometry. The percentage numbers represent the ratio of GP-5 bound cells.

2.3. Assay for the specifically binding affinity of GP-5 to cancer cells

To evaluate the specific affinity of GP-5 binding to GC cells, SGC-7901 cells and HEK293 cells were cultured on cover slips overnight. The slips were fixed with 4% paraformaldehyde at 37 °C for 15 min. After blocking with 1% (w/v) BSA, slips were incubated with 5 μM of the FITC-conjugated GP-5 at 37 °C for 40 min, washed with PBS, and then stained with DAPI (4, 6-diamino-2-phenyl indole), finally observed under a laser scanning confocal microscope (LSCM, Leica, Germany).

2.4. Localization analysis of GP-5 on cancer cells

To directly observe the specifically binding of GP-5 to different cancer and normal cell lines, the cells were cultured on slips in 30 mm dishes, then the slips were stained with Dil membrane probe (Beyotime, Haimen, China), fixed with 4% paraformaldehyde for 15 min at 37 °C, washed three times with PBS, blocked with 1% (w/v) BSA, and finally incubated with FITC conjugated GP-5 (5 μM/dish) for 40 min. After washing, the cells were visualized by LSCM (Leica).

2.5. MTT assay for GP-5 cytotoxicity

The cytotoxicity of GP-5 was evaluated using the MTT assay. The SGC-7901 cells were seeded into 96-well plates with 2.0×10^3 cells/well and divided as the 4 groups (24 h, 48 h,72 h and 96 h) including 6 subgroups with different GP-5 peptide concentration (0.0 mM, 0.03125 mM, 0.0625 mM, 0.125 mM, 0.25 mM, 0.5 mM) respectively. Subsequently, 20 μl of 5 mg/ml MTT in PBS (pH7.4) was added into each well, and then incubated for an additional 4 h at 37 °C. The supernatant was moved, and then 150 μl of DMSO was added to dissolve the formazan crystals formed from MTT with shaking for 10 min. The absorbance was determined using a 96-well plate reader (Biotech, New York, NY) at 570 nm, and this assay was triply repeated.

2.6. Assay for the specifically binding affinity of GP-5 to cancer tissues

Experiments were performed after receiving informed consent and the approval of the Institutional Review Board and Patient Sample Committee of Shaanxi Normal University. Human gastric cancer sample was re-sectioned from the surgery at the First Hospital Affiliated to Xi'an Jiaotong University.

The specifically binding affinity of GP-5 to cancer tissues was investigated using the fluorescence microscopy on the frozen tissue section slides prepared by a regular method. The slides were blocked with 1% (w/v) BSA and then incubated with 5 μM FITC-conjugated GP-5 (5 μM) for 40 min at 37 °C. After being washed three times with PBS, the slides were observed under LSCM.

2.7. Assay for the specifically binding affinity of GP-5 to GC tissue chips

The GC tissue chips were purchased from Alenabio Company (Xi'an, China), and each contains 20 GC samples and 4 GC adjacent normal tissue samples (Tables 1 and 2). The chip slides were deparaffinized with xylene and dehydrated and then submerged into ethylene-diamine-tetraacetic acid (EDTA) antigenic retrieval buffer at 96 °C, finally cooled down for 20 min at room temperature. Subsequently, the treated chip slides were separately blocked with 1% (w/v) BSA and then incubated with 5 μM of the FITC-conjugated GP-5 for 40 min at 37 °C. The chip slides were washed 3 times with PBS, finally imaged under LSCM.

2.8. Statistics

ANOVA test was used as statistical method for the data that is presented throughout this study as mean ± SD for the experiments repeated for 3 times.

3. Results

3.1. Flow cytometry assay for GP-5 specifically binding

The specifically binding affinity of GP-5 to GC cells was further validated by fluorescence-activated cell sorting (FACS) analysis. As shown in Fig. 1, the fluorescence value for binding of FITC-labeled GP-5 to SGC-7901 cells was significantly higher than FITC-labeled control peptide. However, the fluorescence values for the FITC-labeled GP-5 and its control binding to HEK293 cells had shown no significant difference. The results suggested that the FITC-labeled GP-5 targets to SGC-7901 with specifically binding affinity.

3.2. Characterization of the GP-5 binding specificity using fluorescence imaging

Fluorescence imaging was used to the further characterization of the GP-5 binding specificity. As shown in Fig. 2, GP-5 showed the much stronger affinity to SGC-7901 than to HEK293 cells. Furthermore, to observe the specifically binding location of GP-5 on its targeted cells, different cell lines from digestive system were incubated with GP-5. As shown in Fig. 3, FITC-conjugated GP-5 bound to the membrane of SGC-7901 cells specifically and sensitively. The weak binding could be observed on other digestive tract

Table 1
Tissue spot arrays on slide.

	1	2	3	4	5	6	7	8
a	◇	◇	◇	◇	◇	◇	◇	◇
b	◇	◇	◇	◇	◇	◇	◇	◇
c	◇	◇	◆	◆	◇	◇	◆	◆

◇-Malignant tumor.
◆-Adjacent tissue 1.5 cm away from tumor.

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