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Inhibitory effects of anticancer peptide from *Mercenaria* on the BGC-823 cells and several enzymes

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Abstract An anti-cancer peptide was purified from the *Mercenaria* (*Meretrix meretrix* Linnaeus) by the method of chromatography on Sephadex G-25 and FPLC, and its molecular weight was determined to be 3147 Da by the way of MALDI-TOF mass spectrum. The effects of this peptide on human gastric gland carcinoma cells (BGC-823) and their cytoskeletal morphology were investigated. The results showed that the peptide could inhibit the proliferation of BGC-823 cells and obviously destroy the skeletal structures of the cells. When the concentration of the peptide reached 4.0 μg/ml, the inhibition percentage of the cell growth was about 60%. The effects of this anticancer peptide on the activities of superoxide dismutase (SOD), alkaline phosphatase (ALP) and tyrosinase were studied. The results showed that the peptide activated ALP and SOD, but inhibit the tyrosinase activity. When the concentration of the peptide reached to 0.5 μg/ml, the relative activities of SOD, ALP and tyrosinase were determined to be 188.5%, 122.0% and 27.5%, respectively.

Keywords: Anticancer peptide; Human gastric gland carcinoma cell; Superoxidedismutase; Alkaline phosphatase; Tyrosinase; Effect; *Meretrix meretrix* Linnaeus

1. Introduction

Meretrix meretrix Linnaeus is a kind of popular ocean mollusks, the values of which are shown not only in its abundance in nutrition, but also in the use of medical resource. *Mercenaria* has been reported to show eliminate cyst, detoxification and anti-cancer activity [1–3]. Its extract is used in the treatment of cancer for ages. Moreover, *Mercenaria* can be used to decrease the level of blood sugar and blood fat [4,5], and it also has the physiological function of protecting the body cells from mutation and decrepitude [6]. Schmeer et al. [7] extracted a reagent from *Mercenaria* and named it as "*Mercenene*", which was demonstrated to have anticancer activity on L1210, Krebs 2-breast carcinoma, Bittner mammary tumor and so on. However, the molecular characteristic and action mechanism of "*Mercenene*" are unknown. Because the common edible *Mercenaria* is abundant in nature and has never suffered from cancer, this clam is undoubtedly a potential anticancer medicine resource. The aim of the present paper is, therefore, to report the extraction, purification and identification of the anticancer peptide from *Mercenaria*. Furthermore, the effects of this peptide on the cytoskeletal morphology of human gastric gland carcinoma cells (BGC-823) cells are carried out. We also discuss the anticancer mechanism of this peptide by researching its effect on several enzymes relevant to the cancers. The study presented here demonstrates that the peptide from *Mercenaria* has reliable anticancer function, which offers a clue for looking for novel and natural anticancer remedy.

2. Materials and methods

2.1. Materials

Mercenaria was purchased at local market; superoxide dismutase (SOD), alkaline phosphatase (ALP) and mushroom tyrosinase were purchased from Sigma; the haematoxylin and eosin Y (H.E.) were purchased from BDH; the 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was from Fluka. All other reagents were local products of analytical grade. The water used was re-distilled and ion free.

2.2. Extraction and purification

The body from *Mercenaria* (*Meretrix meretrix* Linnaeus) was homogenized and extracted with 25% alcohol for 2 h at 4 °C; then, the extraction was centrifugated at 15 000 × *g* for 30 min (4 °C). The supernatant was gathered and lyophilized to be powder for storage. When purified, the powder was redissolved in water at the concentration of 0.5 g/ml and further chromatographed on Sephadex G-25 column (2.5 × 60 cm), which was eluted with water. The flow rate was controlled at 30 ml/h and 2.5 ml per tube was collected. The fraction with anti-cancer activity was loaded onto flow-performance liquid chromatography (FPLC) with Sephadex G-75 column (16 × 600 mm, 22–44 μm, from Pharmacia, Sweden), pre-equilibrated and eluted with 50 mM Tris–HCl (pH 7.5) buffer. The flow rate was 0.5 ml/min and 1.5 ml per tube was collected. Fractions with anticancer activity were pooled, concentrated and desalted on FPLC with Sephadex G-25 column (1.5 × 50 cm) equilibrated and eluted with water.

2.3. Protein and peptide determination

The concentration of protein and the peptide were determined by the Bradford methods. The molecular weight of the peptide was determined with MALDI-TOF MS, which was performed with a BIFLEX III mass spectrometer (Bruker-Franzen Analytik, Bremen, Germany),

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Abbreviations: BGC-823, human gastric gland carcinoma cells; SOD, superoxide dismutase; ALP, alkaline phosphatase; FPLC, flow-performance liquid chromatography; MTT, 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; H.E., haematoxylin and eosin

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using a 337-nm nitrogen laser. 0.5–1.0 μl of sample (5–20 pmol) and 0.5 μl of α-cyano-4-hydroxycinnamic acid (10 mg/ml in water) were mixed on the target plate and dried with a gentle stream of air. Spectra were obtained in the reflected mode at an accelerating voltage of 19 kV and a reflecting voltage of 23 kV. Deflection of the low mass ions was used to enhance the target protein signal. External calibration (from Bruker) was performed for measurement. Mass determination was averaged from three sample-standard pairs.

2.4. Cell culture

BGC-823 cells were used to test the anticancer activity of the peptide fraction. The cells were cultured in the Roswell Park Memorial Institute tissue 1640 medium containing 1% non-essential amino acid mixture solutions, 10% newborn bovine serum, 100 IU/ml of penicillin and 100 μg/ml of streptomycin with humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Cell numbers were monitored by directly counting with haemacytometer. The inhibitory rate and the variety of cytoskeletal morphology were assessed by the way of MTT [8] and H.E. [9], respectively.

2.5. Determination of enzymes activity

The enzyme activities of SOD, ALP and tyrosinase were determined by the methods of Chen [10], Stewert [11] and Chen [12], respectively.

The effects of the peptide on these enzymes' activities were determined as a percentage of their control activities. All results are expressed as arithmetic means ± S.D. of three independent tests.

3. Results

3.1. Purification of the anticancer peptide from Mercenaria

The crude extraction was applied to the column of Sephadex G-25. The elution profile of peptide was shown in Fig. 1A. There were three protein peaks. Each peak was pooled and tested its effects on BGC-823 cells, respectively. The results indicated that the Peak 3 had strong anticancer activity. The sample of Peak 3 was further purified by FPLC eluted with 50 mM Tris–HCl (pH 7.5). Fig. 1B showed the elution profile and the main peak was pooled for further test.

3.2. Determination of the molecular weight

The molecular weight of *Mercenaria* anticancer peptide was determined to be 3147.41 Da by MALDI-TOF Mass Spectrum (shown in Fig. 2).

3.3. Inhibitory effect of the peptide on BGC-823 cells

The effects of the peptide on the growth of BGC-823 cells were studied. The results (Fig. 3) suggested that the peptide could inhibit the cells at a very low concentration and had a significant dose-dependent-inhibitory effect. When the pep-

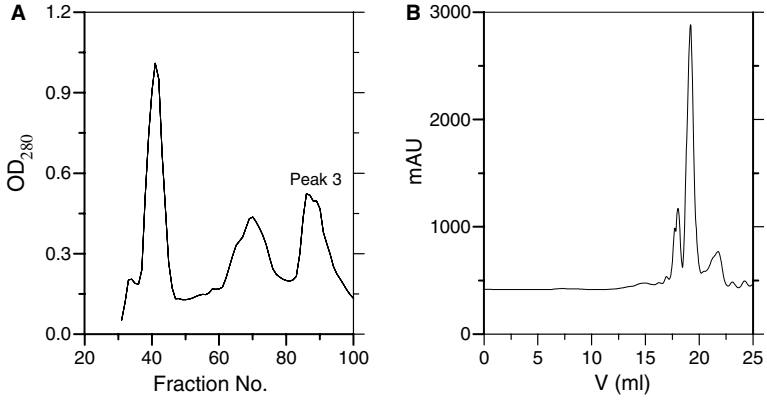

Fig. 1. Column chromatography of anticancer peptide from *Mercenaria* on Sephadex G-25 (A) and on FPLC (B).

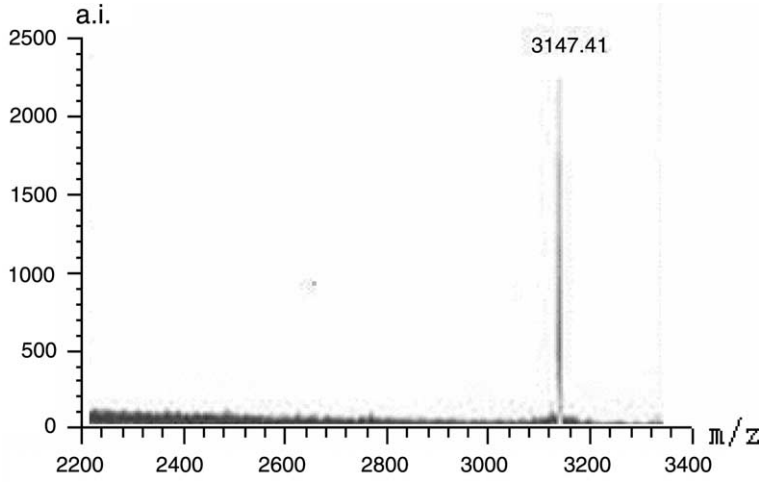

Fig. 2. The determination of the molecular weight of the peptide by MALDI-TOF Mass Spectrum.

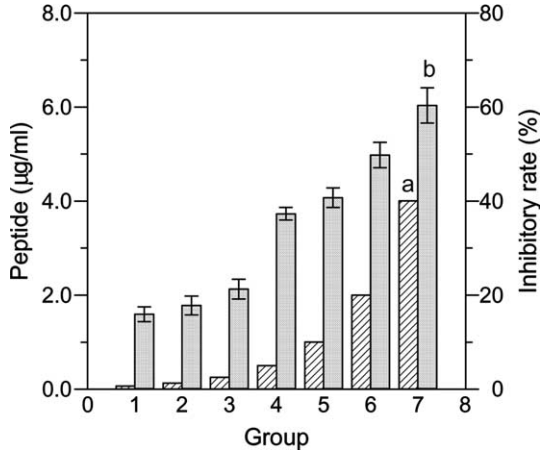

Fig. 3. The inhibitory effect of the peptide on BGC-823 cells. Column (a) shows the concentration of peptide from *Mercenaria* (μg/ml) Column (b) shows the inhibitory percentage (%).

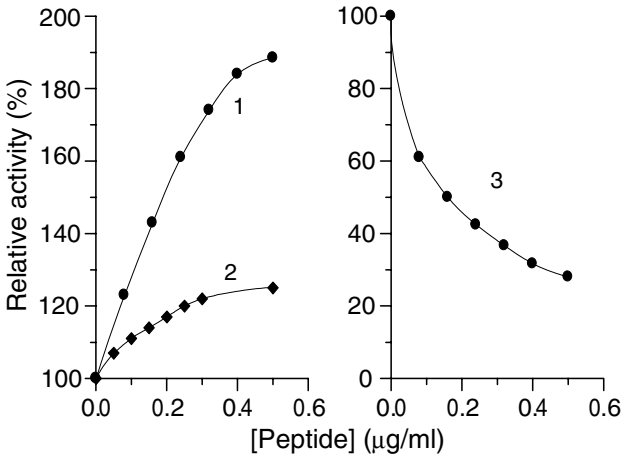

Fig. 5. Effects of the peptide on enzyme activities of SOD (curve 1), ALP (curve 2) and tyrosinase (curve 3).

tide's concentration was 4.0 μg/ml, its inhibitory rate on the growth of BGC-823 cells reached 60%.

The changes of the cytoskeletal morphology of BGC-823 cells treated by the peptide have been investigated (as shown in Fig. 4). The cell's ability of sticking to the bottle surface decreased, and the cell figures bulked and became anomalistic. Moreover, the colouration inside the cells was deeper and the configuration of karyon turned blurred. These results indicated that the peptide could affect the cytoskeletal morphology of BGC-823 cells.

3.4. Effects of the peptide on the activities of SOD, ALP and tyrosinase

The effects of the peptide on the activity of SOD, ALP and tyrosinase were studied, respectively. The results showed in Fig. 5. The peptide could activate SOD (curve 1) as well as ALP (curve 2). With increasing the concentrations of the peptide, the activities of SOD and ALP increased. When the concentration of the peptide reached 0.5 μg/ml, the relative activities of SOD and ALP were 188.5% and 122.0%, respectively. The activation of peptide on SOD activity was more observable than on ALP activity. However, the peptide inhibited tyrosinase with concentration-dependent as shown in Fig. 5 (curve 3). The IC_{50} value, the concentration leading to 50% activity of the enzyme lost, of the peptide was determined to be 0.16 μg/ml.

4. Discussion

In recent years, more and more people paid highly attention to finding novel anti-cancer drugs from natural organism. Anti-cancer peptides have attracted concern recently due to the characteristic of multi-function, high-sensitivity, stability and so on. In this investigation, a peptide from *Mercenaria* was found to strongly inhibit the growth of BGC-823 cells and obviously destroy the cytoskeletal morphology of the cells. Compared with other anti-cancer drugs, *Mercenaria* peptide exhibits higher inhibitory efficiency on BGC-823 cells. Results obtained indicated that *Mercenaria* peptide might be used as a potent anti-cancer drug.

ALP, a kind of membrane-bound metalloenzyme, is related to transport process [13]. Its activity is usually low or repressed in normal stomach tissue and will increase once the cell is malignly transformed [14]. ALP was usually regarded as an important index of differentiation of cancer cells [15]. *Mercenaria* peptide has obvious active effect on ALP, which suggests that the peptide inhibit cancer by the way of accelerating the differentiation of cancer cells.

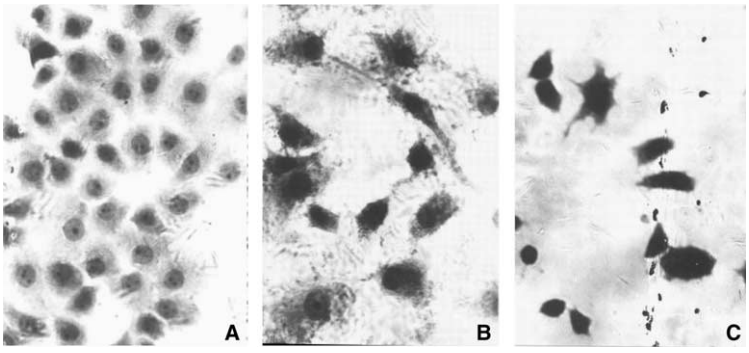

Fig. 4. Effect of the peptide on the cytoskeletal morphology of BGC-823 cells (A) control; (B) treated by the peptide at the concentration of 4.0 μg/ml and (C) treated by the peptide at the concentration of 8.0 μg/ml.

Reactive oxygen species (ROS) joins in generation as well as in malign transformation of cancer cells [16,17]. If compounds can enhance the level of anti-oxidation and clear the ROS in cancer cell, they may inhibit the cell's growth. In our research, *Mercenaria* peptide could activate the SOD, a very important anti-oxidation enzyme, indicating that the peptide inhibited the growth of BGC-823 cells by increasing the activity level of SOD.

Abnormal accumulation of melanin pigments will induce hyperpigmentation. Tyrosinase catalyzes the rate-limiting step for the biosynthetic pathway of melanin pigments. It was reported that *Mercenaria* extraction could evidently inhibit the B16 melanoma and prolong the survival of mice by injecting the extraction into the tumour [18]. Results in our research proved that the peptide could effectively inhibit the activity of tyrosinase, which may be a proof why *Mercenaria* peptide has inhibitory effect on melanoma.

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