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Research paper

Lipid raft biomaterial as a mass screening affinity tool for rapid identification of potential antitumor Chinese herbal medicine☆

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Abstract

Introduction: The quest for novel cancer drugs is one of the most significant studies in natural products, and Chinese herbs with its promising antitumor properties cannot be exempted from this chemotherapeutic bracket. This study was therefore aimed at piloting the use of receptor-rich lipid raft biomaterial as a mass screening tool for rapid isolation of effective antitumor components from some Chinese herbs used in the treatment of diseases consistent with cancer symptoms.

Method: Aqueous crude extracts from 10 different Chinese herbs (Paeonia suffruticosa, Vaccaria segetalis, Terminalia chebula, Trichosanthes kirilowii, Arisaema erubescens, Morus australis, Centella asiatica, Rhematoxylon campechianum, Trachelospermum jasminoides and Taraxacum officinale) were screened using lipid raft silica beads. The identified bioactive products were separated into various fractions (petroleum ether, ethyl acetate, n-butanol and raffinate extracts) with another affinity screening analysis. Conventional MTT bioassay was employed to authenticate the susceptibility of liver cancer cells to the final bioactive isolates.

Results: The results showed that only *Vaccaria segetalis* and *Arisaema erubescens* significantly interacted with the system. The MTT bioassay further confirmed the cytotoxicity of these plants and the data correlated very well with its reported cancer-related activities.

Conclusion: These findings support the reliable application of the lipid raft biomaterial as a prospective mass screening technique for antitumor natural products.

Keywords: Affinity system; Antitumor; Lipid raft; Mass screening; Natural products

1. Introduction

The numerous compounds present in natural products make it very challenging to rapidly and efficiently screen for their effective antitumor parts on a large scale. The available conventional methods like the different forms of chromatography, coupled with cell viability/cytotoxicity assays have not been able to fully address the problem. There is also mostly no biological interaction between the analyte and the chemical screening system due to the fact that such separation is always based on the physicochemical properties [1]. Additionally, the use of bio-directed activity comes with its own disadvantages such as time-constraint, cost implications and in certain situations unexpected negative outcomes. Now several affinity screening systems like immobilized DNA chromatography [2], immobilized plasma protein chromatography [3,4], immobilized liposome chromatography [5] and immobilized biomembrane chromatography [6–8] have evolved and continued to be employed in isolating bioactive compounds. Technically, these systems are by far better than the traditional methods; however, there is the need for

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further development and enhancement of these affinity systems to directly link the diseased condition to its associated anti-potential agent. This therefore provides a great opportunity for us to explore novel screening tools to maximize the antitumor prospects in natural products.

In previous studies a giant attempt was made to provide a cell membrane-based model in isolating bioactive compounds. Thus, He et al. [9,10] first applied the technique of immobilized biomembrane chromatography for screening active components from traditional Chinese medicine (TCM). In the study, immobilized rat vascular cell membrane serving as a stationary phase was successfully used to investigate the bioactive components of vasodilatation in TCMs such as *Radix Angelicae Dahuricae*, *Rhizomza Seu Radix Notopterygii*, *Radix Glehniae* and *Fructus Cnidii* [11]. However, setbacks like poor recognition and identification of different receptor isoforms in the cell membrane chromatography (CMC) have impeded its further development. Providing an efficient screening system, the subtype target receptors should be richly expressed in the stationary-phase. Besides, the stability of the ligand which determines the durability of the column and the cost of the process should meet acceptable standards. However, the life-span of most CMC columns could last for only 3 days or just 1 week under a continuous usage [11]. Therefore, further studies are needed to improve the stimulus–receptor interaction-based chromatography.

Chinese herbal medicine (CHM) has pharmacologically exhibited unimaginable antitumor properties and become a cynosure of modern medicine [12,13]. However, very few chemical screening systems have successfully unearthed its great potential for an effective cancer therapy. Inspired by this development, the lipid raft stationary chromatography (LRSC) previously established by our group was employed on a larger scale to identify prospective antitumor plant candidates among Chinese herbs noted for their use in treating cancer-like symptoms; however, with limited detailed scientific information on the reported antitumor activities. Basically, the lipid rafts are specific microdomains enriched in dynamic assemblies of cholesterol and sphingolipids on the plasma membrane of eukaryotic cells [14]. Many studies have suggested that they play a significant role in a wide range of major biological processes such as signal transduction pathways, apoptosis, cell adhesion and migration, synaptic transmission, organization of the cytoskeleton and protein sorting during both exocytosis and endocytosis [15–17].

In this study, the lipid raft was isolated from cancer cell membrane and immobilized on an activated silica support to serve as a stationary phase. Previous studies had shown that the LRSC could easily single out potential antitumor effective components and even significantly shortens the isolation process as compared to the conventional methods. It is also cost effective and less time consuming. Additionally, several natural products could effectively be screened with the affinity system. The biomaterial further has the flexibility of being developed from other cancer cell types and receptor-rich lipid rafts from different parts of the biomembrane. These undoubtedly could cater for similar natural products which might escape a particular biomaterial. Here, we report the use of the novel lipid raft biomaterial chromatography as a mass screening system for rapid isolation of potential antitumor CHM. The biomaterial with sufficiently expressed tropomyosin-related kinase A (TrkA) receptor was prepared as described in previous studies [18,19]. Then ten (10) different aqueous Chinese herbal extracts were prepared and analysed using the LRSC for their antitumor prospects. Further isolation was carried out to partition the bioactive extracts into different fractions (Petroleum ether, ethyl acetate, n-butanol and aqueous residue extracts) and subsequently run on the affinity system. The MTT bioassay was used to confirm the antitumor effect of the final bioactive LRSC isolates.

2. Methods

2.1. Plant materials

In this study, Chinese herbs used in treating symptoms consistent with cancers, but with less detailed scientific report regarding its antitumor activities were selected based on an extensive investigation carried out within 6 months. The ten selected plants (*Paeonia suffruticosa* Andrews, *Vaccaria segetalis* (Neck), *Terminalia chebula* Retz. *Trichosanthes kirilowii* Maxim, *Arisaema erubescens* (Wall.) Schott, *Morus australis*. Poir., *Centella asiatica*, *Rhematoxylon campechia*num, *Trachelospermum jasminoides* (Lindl.) Lem and *Taraxacum officinale* Webb.) were identified by consulting common scientific books and published data on the popular uses of these plants. The raw materials were bought from Yancheng Chinese herbal medicine Co., Ltd (Sichuan, China) and authenticated by Professor Huan Yang, School of Pharmacy, Jiangsu University. The terminologies used to search for the information in the databases include Chinese herbal medicine, antitumor, cancer treatment and medicinal uses. There was no limitation on the language of publication, however most significant data were published in English and Chinese.

2.2. Other materials

HepG-2 human liver cancer cell lines were obtained from Cell Bank of Academy of Science (Shanghai, China). Foetal bovine serum and Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco Company (Invitrogen Co., Carlsbad, CA, USA). 5-Fluorouracil was supplied by Jin Yao Amino Acid Co., Ltd. (Tianjin, China). (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and trypsin were purchased from Beyotime Institute of Biotechnology (Jiangsu, China). Parenzyme and gemcitabine were obtained from Sigma (St. Louis, MO, USA). Tris–HCl was purchased from Shenergy Biocolor Bioscience and Technology Company (Shanghai, China). Dialysis bags with molecular cut off of 3,500 Da were obtained from Green Bird Technology Development Co., Ltd (Shanghai, China). Silica (50 μm, 60 Å) was purchased from Sepax Technologies, Inc. (Delaware, USA). Lestaurtinib and gemcitabine were purchased from LC Laboratories (Woburn,