



Ligand fishing with cellular membrane-coated cellulose filter paper: a new method for screening of potential active compounds from natural products

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Abstract

Ligand fishing is a widely used approach for screening active compounds from natural products. Recently, cell membrane (CM) as affinity ligand has been applied in ligand fishing, including cell membrane chromatography (CMC) and CM-coated magnetic bead. However, these methods possess many weaknesses, including complicated preparation processes and time-consuming operation. In this study, cheap and easily available cellulose filter paper (CFP) was selected as carrier of CM and used to fabricate a novel CM-coated CFP (CMCFP) for the first time. The type of CFP was optimized according to the amount of immobilized protein, and the immobilization of CM onto CFP by the insertion and self-fusion process was verified by confocal imaging. The CMCFP exhibited good selectivity and stability and was used for fishing potentially active compounds from extracts of *Angelica dahurica*. Three potentially active compounds, including bergapten, pabulenol, and imperatorin, were fished out and identified. The traditional Chinese medicine systems pharmacology database and analysis platform was used to build an active compound-target protein network, and accordingly, the gamma-aminobutyric acid receptor subunit alpha-1 (GABRA1) was deduced as potential target of CM for the active compounds of *Angelica dahurica*. Molecular docking was performed to evaluate the interaction between active compounds and GABRA1, and bergapten was speculated as a new potentially active compound. Compared with other methods, the fishing assay based on CMCFP was more effective, simpler, and cheaper.

Keywords Ligand fishing · Cellular membrane-coated cellulose filter paper · Active compounds · Network pharmacology

Introduction

Natural products are the constituent components of plants, animals, insects, marine organisms and microbes, or their

metabolites, as well as many endogenous chemical constituents from animals. Traditional Chinese medicine (TCM) plays an important role in usage of natural products for treatment and prevention of various diseases, especially in Eastern

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Asian countries [1, 2]. The screening of drug candidates from natural products is effective and economical. Hence, many new drugs originate from natural products and are widely used in clinical treatment. From natural products to innovative drugs, the critical step is the identification of biologically active lead compounds. The typical identification process usually includes chemical separation and biological assay. Many inactive compounds are simultaneously isolated along with active compounds during separation steps, which make the identification process labor intensive and time consuming [3, 4]. Therefore, the development of innovative approaches for rapidly and efficiently separating active ingredients from natural products is particularly important.

One of the most valid approaches is affinity-based screening assay, also called ligand fishing, which relies on the principle of macromolecular target-ligand binding, including antigen-antibody, receptor-ligand, and enzyme-inhibitor/activator [5]. Hence, various types of macromolecular target are applied to affinity-based assay, for example receptor, enzyme, transport protein, and cell membrane [6]. Ligand fishing can be classified into two categories, including on-line and off-line models. The on-line mode ligand fishing has been performed on biochromatography, capillary electrophoresis, and bio-reactor [7–10], which can carry out separation and analysis at the same time. But, the immobilization of target molecule on the stationary phase is complicated and time consuming, for example, preparation of APTES-decorated HepG2 cancer stem cell membrane chromatography requires about 4 days [7]. Furthermore, a large number of inactive compounds could contaminate and block the pipeline of instrument, especially in detection system.

In off-line mode, affinity selection and analysis of active compounds are two separate steps. Active compounds are first trapped by immobilized target molecule on the stationary phase, and then washed off and subjected to analysis [6]. Affinity selection just needs less pretreatment of samples, and analysis can be performed on various instruments to meet the different requirements of natural products. The bound compounds can be separated from the unbound compounds through solid-phase extraction, dialysis, ultrafiltration, and size-exclusion chromatography [11–15]. Hence, target molecules are immobilized on various stationary phases, including magnetic beads, quantum dots, hollow fibers, and nanotubes [3, 13–17]. Yet, these strategies possess respective weakness, including complicated fabrication process, time-consuming operation, and requirement of additional equipment.

Cell membrane (CM) includes numerous membrane proteins and ion channels, most of which are the targets of approved drugs [18]. CM has been widely applied in ligand fishing, especially cell membrane chromatography (CMC) [19], which has been proven to be useful for screening active components from natural products. But the preparation process of CMC column is complicated and time consuming [20].

CM of red blood cell has also been used to directly fish potential active components from *Angelica sinensis* [21]. However, the method needs multiple centrifugations to separate CM-active component complexes from inactive components and CM from active components, which are tedious and time consuming. Recently, our group has prepared CM-coated magnetic beads for the screening of potentially active components [22]. Though the process is simple and time saving, the method requires external magnets.

Membrane extraction has been used widely in separation and purification, because it reduces consumption of solvent and facilitates the extraction procedure, in which the separation can be performed by tweezers [23, 24]. However, membrane extraction has never been used for fishing ligands from natural products. Recently, our group has proposed an insertion/self-fusion mechanism of CM immobilization, which is that CMs insert into the pore canals of porous silica beads, resulting in membrane immobilization [25]. The cellulose filter paper (CFP) possesses predetermined pore canals, which can allow cell membranes insert. In addition, the hydroxy of cellulose contributes to the formation of strong intermolecular hydrogen bonds between CFP and CM, and the cellulose fibers present a moderately hydrophobic surface, which is beneficial for immobilization of hydrophobic lipid bilayer of CM [26]. In this work, a novel cell membrane-coated cellulose filter paper (CMCFP) was fabricated for the first time and used for fishing potentially active compounds from a natural product (*Angelica dahurica*). The active compounds, including bergapten, pabulenol, and imperatorin, were fished out and studied by network pharmacology method.

Experimental

Chemicals and materials

CFPs with different average pore sizes (see Electronic Supplementary Material (ESM) Table S1) were obtained from Whatman (Germany). 1,1'-Dioctadecyl-3,3',3',3'-tetramethylindocarbocyanine (DiI, red fluorescent probe of CM) was obtained from Beyotime Biotechnology Co. LTD (Shanghai, China). Fluorescein isothiocyanate isomer (FITC) and verapamil were bought from Sigma-Aldrich (Shanghai, China). Acetonitrile (HPLC grade), dimethyl sulfoxide (DMSO), ethylenediaminetetraacetic acid disodium salt (EDTA-2Na), methanol, acetic acid, sodium chloride (NaCl), thiourea, and dipotassium phosphate (Na_2HPO_4) were obtained from Concord Technology Co. Ltd (Tianjin, China). *Angelica dahurica* was purchased from LBX Pharmacy Co. LTD (Tianjin, China). Alsever's solution (red cell storing solution) was obtained from Solarbio Science and Technology

LTD (Beijing, China). BCA protein kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Instrumentation

Cell disruption was achieved by a KQ-200VDE supersonic cleaner (Kunshan Supersonic Equipment Co. LTD, Kunshan, China). The protein concentration of CM was determined by a UV-3100 UV spectrophotometer (Hitachi, Tokyo, Japan) at the wavelength of 562 (1.5 nm of slit width) nm combined with BCA protein kit. The images were measured with an FV1000 confocal microscope (Olympus, Tokyo, Japan). Ultrapure water was produced with a Milli-Q water purification system (Millipore, Bedford, USA). An Agilent 1260 infinity HPLC system (Agilent Technologies, Palo Alto, USA) was used for qualitative analysis of potentially active compounds. The structures of active compounds were obtained from Agilent 6210 ESITOF LC/MS system. The chromatographic separation was performed on an Eclipse XDB C18 column (250 × 4.6 mm, I.D., 5 μm) supplied by Agilent Technologies.

Preparation of CMCFP

CM of red blood cell (RBC) was prepared as previous report with minor modification [22]. The procedure about rabbit was approved by Animal Ethics Committee, Tianjin Medical University, Tianjin, China. All the experiments on rabbits were performed in compliance with the guide of care and use of laboratory animals. In brief, blood was obtained from the heart of rabbit and stored in Alsever's solution before usage. After centrifugation of blood sample at 1500g for 15 min at 4 °C, the obtained pellets were resuspended in normal saline and centrifuged at 6000g for 5 min at 4 °C (repeat three times). The cell pellet was diluted by 50-fold with a low osmotic solution (0.1 mmol/L EDTA-2Na, 5 mmol/L Na₂HPO₄, pH = 8.0), and then sonicated for 5 min at 4 °C to disrupt the cells. The resulting homogenate was centrifuged at 15,000g for 40 min at 4 °C, and then resuspended in normal saline (repeat three times). The suspension of CM was stored at 4 °C before usage.

CFPs were cut into rectangle (5 × 3 mm, 5 × 5 mm, 10 × 5 mm, and 10 × 10 mm) and soaked in 500 μL of CM suspension in 1.5-mL EP tubes. The mixed solution was shaken gently for 30 min at 4 °C and stored at 4 °C overnight. Then, the CMCFP was pulled out by a tweezer and washed with normal saline (repeat three times) to remove the unfixed CMs. The protein concentration of CM on CFP cannot be determined directly, because some CFPs could result in degeneration of BCA reagent. Therefore, the protein concentration of unfixed CMs was determined by BCA kit and used to calculate the protein concentration of CMCFP.

Preparation and confocal imaging of FITC/DiI double-labeled CMCFP

The FITC-labeled proteins of CM were prepared as previous report [22]. In brief, 10 μL of FITC solution (dissolved in DMSO, 1 mg/mL) was slowly added into 1 mL of suspension of CM. The mixed solution was protected from light and stored for 8 h at 4 °C. The FITC-labeled proteins of CM were collected by centrifugation of the mixed solution at 1500g for 15 min at 4 °C and resuspended in normal saline (repeat three times) to remove the excess FITC. The DiI-labeled phospholipid bilayer of CM was synthesized in an identical manner to the FITC-labeled proteins of CM, but with DiI instead of FITC. The FITC/DiI double-labeled CMs were immobilized onto the CFP, according to the description in the “Preparation of CMCFP” section. The FITC/DiI double-labeled CMCFP was stored at 4 °C before usage. The FITC-labeled proteins of CMCFP were observed on an FV1000 confocal microscope with 480 nm of excitation wavelength and 542 nm of detection wavelength (× 10 objective, green pseudocolor used for showing luminescence). The DiI-labeled phospholipid bilayer of CMCFP was also observed with 540 nm of excitation wavelength and 633 nm of detection wavelength (× 10 objective, red pseudocolor used for showing luminescence).

Ligand fishing by CMCFP

A mixture of verapamil (100 μmol/L) and thiourea (100 μmol/L) was used to evaluate the selectivity of CMCFP. CMCFP was co-incubated with 1 mL of mixture for 30 min at 37 °C. Then, the CMCFP was removed by a tweezer and washed with normal saline, until no verapamil was detected in washing solution. Next, the CMCFP was eluted with 20% acetic acid solution. Finally, the eluent was freeze-dried, and the residue was redissolved and analyzed by HPLC. Verapamil and thiourea were analyzed on an Agilent Eclipse XDB C18 (4.6 × 250 mm, 5 μm). The mobile phase was acetonitrile/0.1% formic acid solution (40:60, v/v), and detection wavelength was set at 265 nm. Column temperature and flow rate were set at 30 °C and 0.8 mL/min, respectively.

Extraction of chemical constituents from *Angelica dahurica* was performed according to the previous report [22]. Briefly, 10 mg of crude herbs (*Angelica dahurica*) was extracted with 80 mL of methanol for 0.5 h. The methanol was removed by vacuum evaporation. Subsequently, the chemical constituents were redissolved in 10 mL of deionized water. The extracted solution was filtered through a 0.22-μm membrane filter and stored at 4 °C before ligand fishing. Ligand fishing from TCM (*Angelica dahurica*) was performed in an identical manner to the above description but with the extracted solution instead of mixture (verapamil and thiourea) solution. A control ligand

fishing experiment was also performed using normal saline instead of extracted solution.

Network construction and molecular docking

Network construction was performed as follows: (1) the active compounds obtained from ligand fishing were identified by LC-MS; (2) the target proteins of active compounds in *Homo sapiens* were retrieved from the traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP) [27]; the active compound-target protein network was constructed and analyzed in the network visualization software Cytoscape [28].

Molecular docking was performed by employing the docking software systemDock (<http://systemsdock.unit.oist.jp/iddp/home/index>). After the structure of the target protein, which was obtained from the Protein Data Bank (<http://www.rcsb.org/>), was first input into, and then the active compound was input into, the binding portfolio of compound-protein pairs was obtained [29, 30].

Results and discussion

Preparation and characterization of CMCFP

RBC has been used for ligand fishing in previous reports [21, 22], due to their easy-to-operate. So, RBC was selected as model CM in this work. The cheap and easily available CFP has predetermined pore canals for inserting CMs. Besides, the hydrogen bond and hydrophobic interaction between CFP and CM are beneficial for immobilization of CM. Thus, CFP was selected as carrier of CM. The preparation process of CMCFP is shown in Fig. 1. The RBC of rabbit was first broken by hypotonic swelling method, and then the obtained CMs were fixed to the CFP by physical adsorption. The ligand fishing process included four steps: incubation for binding active compounds, washing for removing inactive compounds, separation by tweezers, and elution for releasing active compounds. The active compounds were further analyzed by HPLC and LC-MS. The proposed ligand fishing method is simple, low cost, and easy to implement.

The previous report has demonstrated that the immobilization of CMs on porous silica beads can be performed by insertion/self-fusion mechanism [25], and the pore size of carrier has important influence on the immobilization. Therefore, CFPs with different average pore sizes (ESM Table S1) were used for the immobilization of CMs. CM fragments could be inserted into large diameter channels, but not into small diameter channels. When the pore diameter was too large, the fragments might fall off during ligand fishing process. As shown in Fig. 2, the maximum amount of immobilized protein was observed on grade 2 CFP and was 0.162 ± 0.021 mg/cm²,

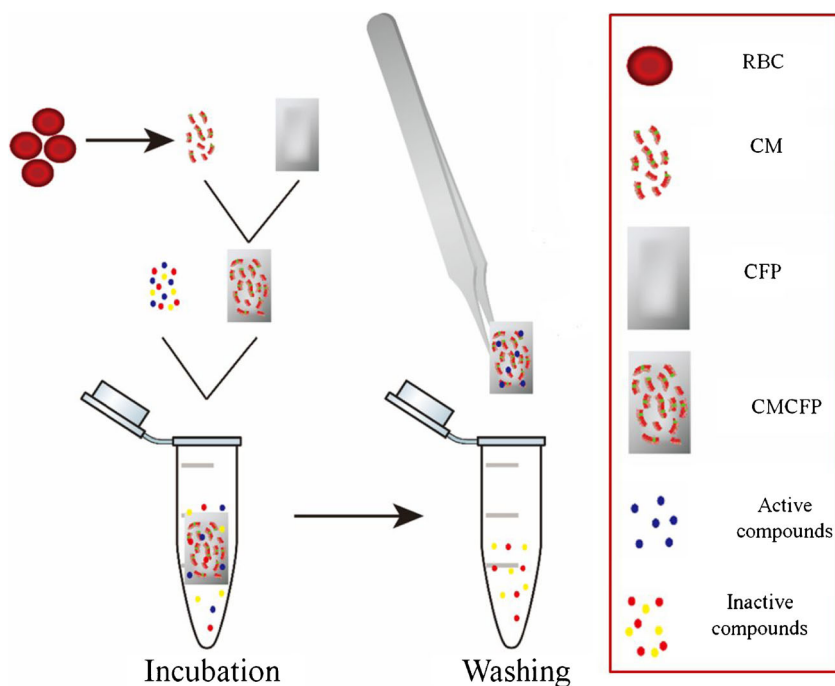
indicating the optimal type of CFP. This result indicated that most CM fragments were about 8 μ m in diameter. The reproducibility of different CMCFPs by five operators was various with relative standard derivation (RSD) between 13.0 and 31.5% ($n = 5$), and the best reproducibility (13.0%) was observed on grade 2 CFP. This may be due to that the size distribution of CM fragments and the pore size distribution of CFP were not uniform. Hence, the CMCFP prepared by grade 2 CFP was selected for the following experiments.

The scanning electron photographs (ESM Fig. S1) exhibited the morphologies of CFP and CMCFP. Compared with CFP (ESM Fig. S1A), the CMCFP had a lot of light blobs (ESM Fig. S1B), which indicated the immobilization of CMs on CFP. To further confirm the immobilization of CMs on CFP, FITC/DiI double-labeled CM was immobilized onto the CFP and observed by confocal microscopy. The CFP was transparent (Fig. 3A1), and without green (Fig. 3A2) and red (Fig. 3A3) fluorescence. In contrary, FITC/DiI double-labeled CM is fragmented (Fig. 3B1), and with green (Fig. 3B2) and red (Fig. 3B3) fluorescence. After FITC/DiI double-labeled CM was immobilized onto the CFP, the transparency of CMCFP reduced sharply (Fig. 3C1), which demonstrated the CMs had inserted into the predetermined pore canals of CFP. In addition, significant green fluorescence (Fig. 3C2) was visible on CMCFP, indicating the distribution of FITC-labeled CM. Similarly, red fluorescence (Fig. 3C3) indicated the distribution of DiI-labeled CM on CMCFP. These results verified that the CM was successfully immobilized onto the CFP through the insertion and self-fusion process, which was similar with previous report [25].

Selectivity and stability of CMCFP

Verapamil is a dihydropyridine calcium channel blocker, which can bind with calcium channel protein on CM surface [31], and thiourea has no mutual effect with CM. Upon immobilization of CM, the resultant CMCFP could be devoted to selectively fish CM-interactive compound. Thus, a mixture of verapamil and thiourea was chosen as model sample for identification of the selectivity of the ligand fishing process [32, 33]. As shown in Fig. 4, the verapamil and thiourea were both detected and separated by HPLC (Fig. 4a). In the last washing solution (Fig. 4b), no peak of verapamil and thiourea was found, which suggested that the inactive compound (thiourea) was completely washed away and the active compound (verapamil) firmly adsorbed on CMCFP. Only the active compound (verapamil) was eluted from CMCFP and found in chromatogram (Fig. 4c), which indicated that the prepared CMCFP could selectively fish verapamil due to the specific interaction between verapamil and calcium channel protein. To further verify the specificity of CMCFP, bare CFP was also used to perform the fishing process. Both verapamil and thiourea were not found in the eluent from bare CFP (Fig.

Fig. 1 Immobilization of cellular membrane (CM) (red blood cell, RBC) on cellulose filter paper (CFP), and fishing process of active compounds by cellular membrane-coated cellulose filter paper (CMCFP)



4d). The enrichment factor of fishing assay based on CMCFP was 50, and the limit of detection with the signal-to-noise ratio of 3:1 for verapamil was 4 pmol/mL. These results demonstrated that resultant CMCFP could be applied for selective fishing of active compounds, which specifically interacted with CM, from a mixture.

The batch to batch reproducibility of CMCFP by one operator was evaluated by the amount of immobilized protein. As a result, intra-day ($n = 5$) and inter-day ($n = 8$) RSDs of 8.5% and 12.5% were obtained, suggesting good reproducibility of preparation. In addition, the batch to batch repeatability of fishing capacity of CMCFP was also evaluated by fishing verapamil solution. The CMCFP was used only once. The intra-day ($n = 5$) and inter-day ($n = 8$) RSDs of 12.1% and 19.2% were obtained for the fishing capacity, suggesting

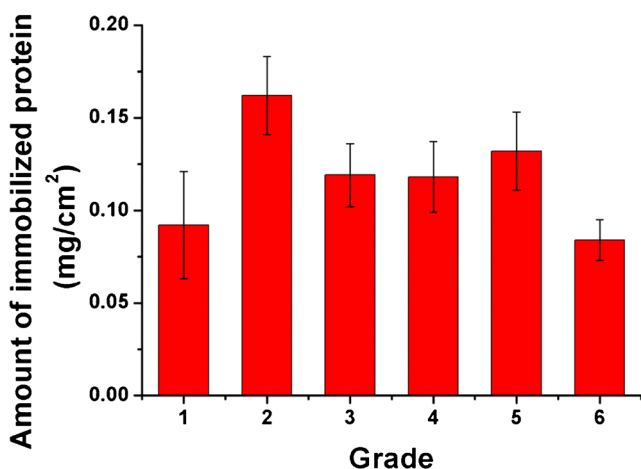


Fig. 2 Amount of immobilized protein on different CMCFPs

acceptable repeatability of application. The fishing capacity of CMCFP was 12.6 nmol/cm² ($n = 15$), indicating that calcium channel protein may be presented at the CMCFP. Calcium channel protein is one kind of membrane proteins laid on the CM of RBC, so the fishing capacity in this study was lower than other fishing assay [34, 35]. The problem of low fishing capacity could be solved by immobilization of receptor-high-expression cell. The effect of CFP size on CM load was also optimized. As shown in Fig. S2 (see ESM), there was no obvious change about the amount of immobilized protein on CM, with the increase of CFP size or reduction of CM concentration. This result indicated that the CM was adequate in the process of immobilization of CM on CFP. In addition, the fishing capacity could be enhanced by increasing CFP size.

The reusability of CMCFP for fishing assay was also investigated by fishing verapamil solution. The regeneration of CMCFP was achieved by repeatedly soaking in normal saline to remove the residual of acetic acid derived from eluent. As shown in Fig. S3 (see ESM), the binding capacity of CMCFP sharply decreased with increase of the regeneration times, and no verapamil was fished out after five cycles. This result indicated that the biological activities of CM immobilized on CMCFP deteriorated gradually, or the immobilized CM fell out from CFP, due to the acetic acid derived from eluent. Thus, assembled CMCFP could only be used once. The storage temperature and time had important effect on the performance of CMCFP. The binding capacity of CMCFP decreased gradually when stored at 4 °C for 48 h (Fig. 5A), but had no obvious change when -20 °C of storage lasted 168 h (Fig. 5B). The amount of immobilized protein on CMCFP possessed similar trend (ESM Fig. S4). The result

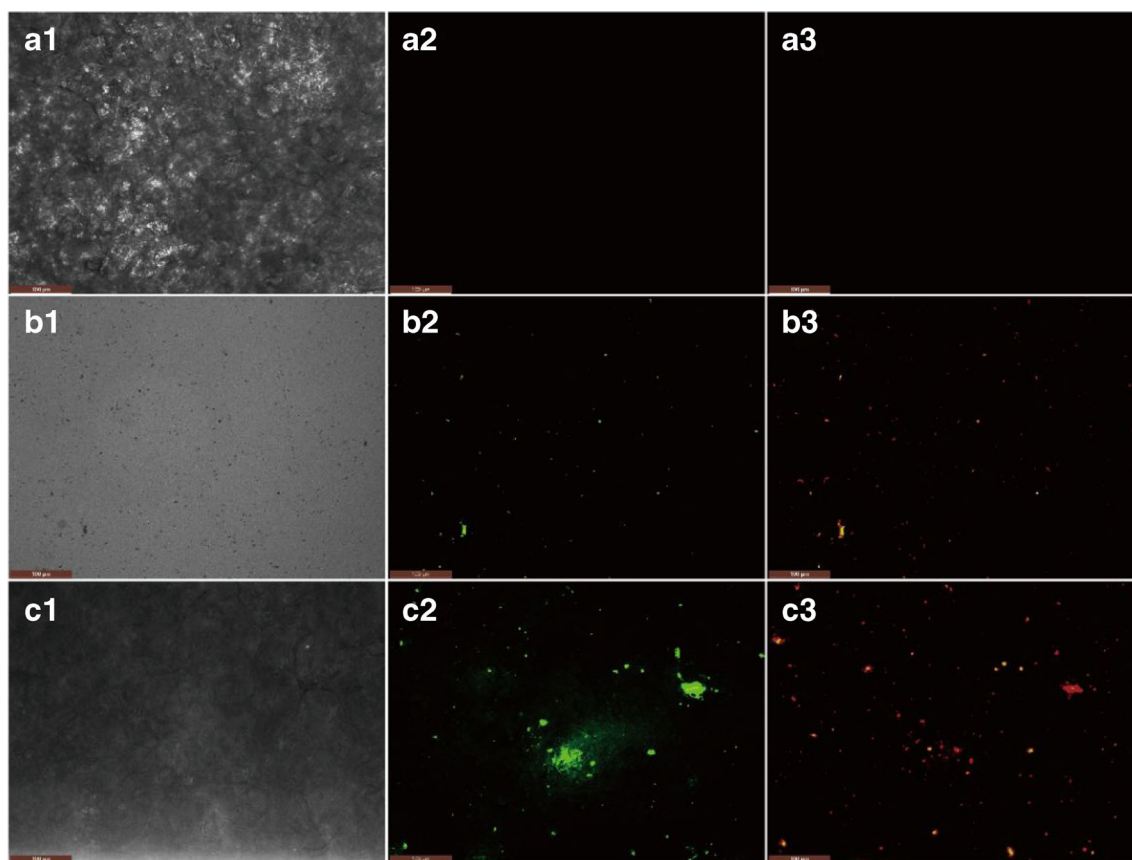


Fig. 3 Confocal microscopic images of CFP (A), suspension of FITC/DiI double-labeled CM (B), and FITC/DiI double-labeled CMCFP (C): (A1, B1, C1) the transmitted light images; (A2, B2, C2) the green fluorescence images of activated carries with 480 nm of excitation wavelength and

542 nm of detection wavelength to indicate the FITC-labeled proteins; (A3, B3, C3) the red fluorescence images of activated carries with 540 nm of excitation wavelength and 633 nm of detection wavelength to indicate the DiI-labeled phospholipid bilayer

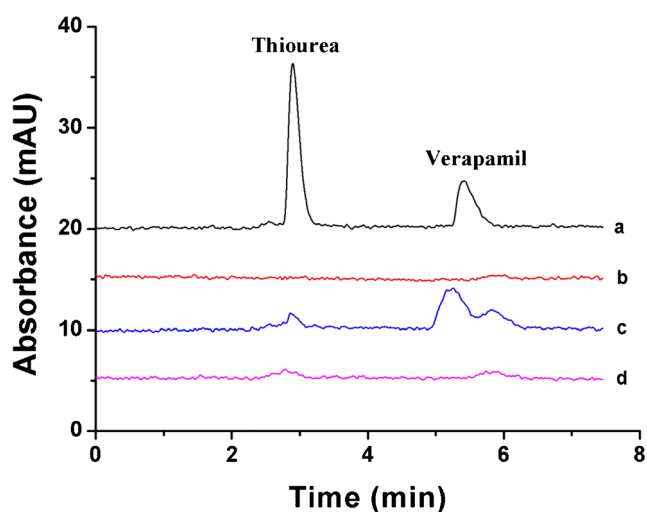


Fig. 4 HPLC results of the mixture of verapamil and thiourea (a); the last washing solution after washing step (b); the eluent of CMCFP after incubation with the mixture of verapamil and thiourea (c); the eluent of bare CFP after incubation with the mixture of verapamil and thiourea (d). Column, Agilent Eclipse XDB C18 (4.6 × 250 mm, 5 μm); column temperature, 30 °C; detective wavelength, 300 nm; mobile phase, acetonitrile/0.1% formic acid solution (40:60, v/v); flow rate, 0.8 mL/min

demonstrated that low-temperature storage was beneficial to the stability of immobilized CMs. Therefore, the CMCFP should be stored at −20 °C to avoid the gradual decrease of binding capacity.

Fishing from *Angelica dahurica*

Calcium channel protein on CMCFP can selectively fish its ligand (verapamil), so the resultant CMCFP including many other membrane proteins could be used for screening active compounds, which can interact with membrane proteins. *Angelica dahurica* was selected as the model natural product and analyzed by HPLC according to previous report [36]. Compared with chromatograms of extract of *Angelica dahurica* (Fig. 6a), the chromatograms of the eluate from CMCFP, which incubated with extract of *Angelica dahurica*, exhibited three intensive peaks (Fig. 6b). The increased peaks and missing peaks may represent active and inactive compounds in *Angelica dahurica*, respectively. Bare CFP was also used to incubate with extract of *Angelica dahurica*. No peak (active compound) was found in the eluent from bare CFP (Fig. 6c). These results indicated that ligand fishing was based

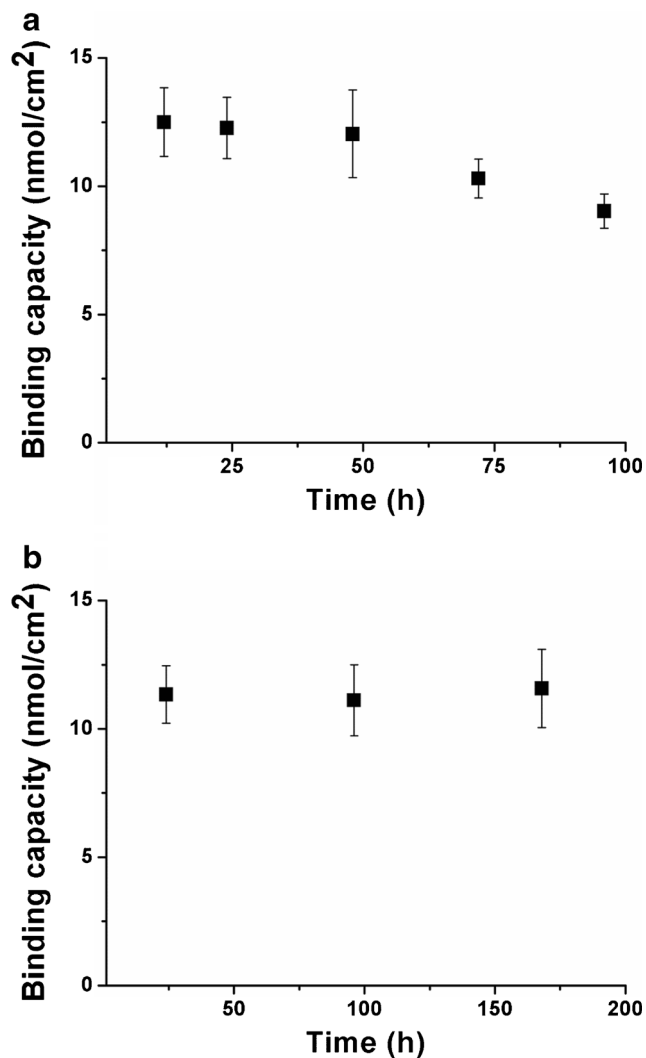


Fig. 5 The binding capacities of CMCFP for fishing verapamil after storage at 4 °C (A) and -20 °C (B) for different times

on the specific interaction between active compounds derived from *Angelica dahurica* and CM immobilized on CFP. The fishing result was according to previous report [22].

The three active compounds (Fig. 6b), including bergapten, pabulenol, and imperatorin, were identified by high-resolution MS (ESM Fig. S5) and compared with standard substance, and their chemical structures are exhibited in Fig. 6. The molecular weights of peaks 1 (bergapten) and 2 (pabulenol) were 216.19 and 296.28, respectively, which were same with previous report [37]. Imperatorin (peak 3) could induce vasodilatation possibly via inhibiting voltage-dependent calcium channel and receptor-mediated Ca²⁺ influx and release [38]; therefore, it could be fished out by interacting with calcium channel protein on CMCFP. The three active compounds had common parent nucleus psoralen, so bergapten and pabulenol may also interact with the calcium channel protein or other membrane proteins. Detailed pharmacological activity of three active compounds should be investigated.

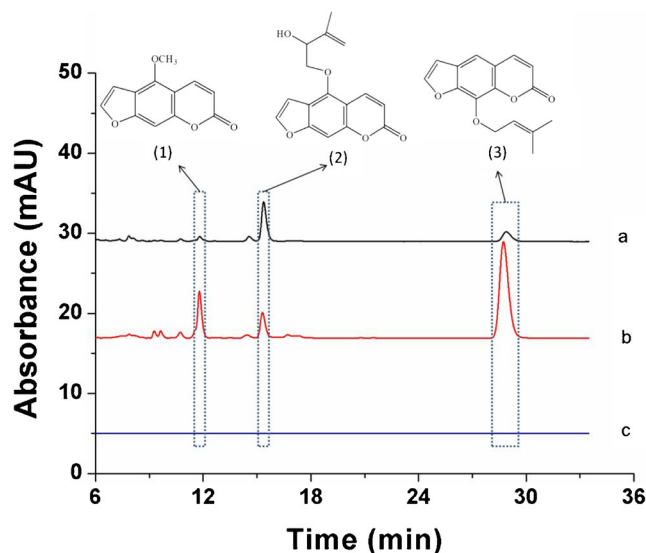


Fig. 6 HPLC result of the *Angelica dahurica* extract (a); the eluent of CMCFP after incubation with the extract (b); the eluent of CMCFP after incubation with normal saline (c). (1) bergapten, (2) pabulenol, and (3) imperatorin. Column, Agilent Eclipse XDB C18 (4.6 × 250 mm, 5 μm); column temperature, 30 °C; detective wavelength, 300 nm; mobile phase, acetonitrile/0.1% formic acid solution (40:60, v/v); flow rate, 0.8 mL/min

Network construction and molecular docking

TCMSP is a unique framework of systems pharmacology platform, which includes all the 499 Chinese herbs recorded in Chinese Pharmacopoeia, and 29,384 ingredients, 3311 targets, and 873 associated diseases [27]. The three active compounds were retrieved by TCMSP. According to the refined results (ESM Table S2), the active compound-target protein

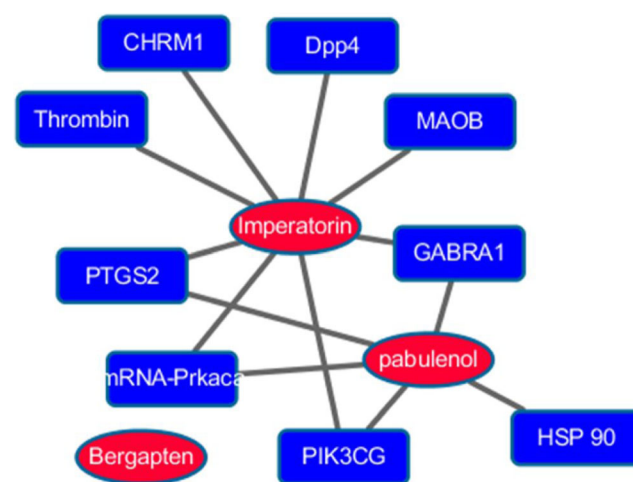


Fig. 7 The active compound-target protein network. Muscarinic acetylcholine receptor M1 (CHRM1), prostaglandin G/H synthase 2 (PTGS2), gamma-aminobutyric acid receptor subunit alpha-1 (GABRA1), dipeptidyl peptidase IV (Dpp4), hosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, gamma isoform (PIK3CG), mRNA of PKA catalytic subunit C-alpha (mRNA-Prkaca), amine oxidase (flavin-containing) B (MAOB), and heat shock protein 90 (HSP 90)

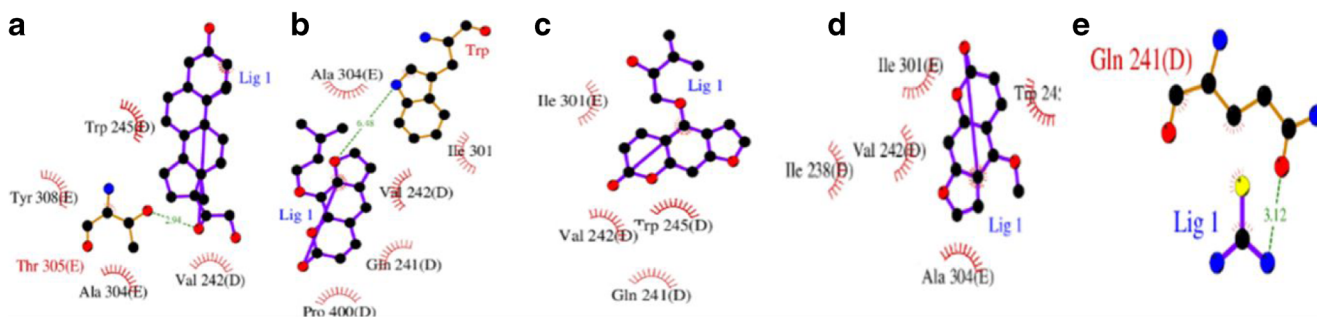


Fig. 8 Visualize interaction in 2D by molecular docking between GABRA1 and native ligand (a), imperatorin (b), pabulenol (c), bergapten (d), and thiourea (e)

network was constructed and is shown in Fig. 7. No target for Bergapten was retrieved. In contrary, there were 8 and 5 targets from imperatorin and pabulenol, including 4 repetitive targets: prostaglandin G/H synthase 2 (PTGS2), gamma-aminobutyric acid receptor subunit alpha-1 (GABRA1), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, gamma isoform (PIK3CG), and mRNA of PKA catalytic subunit C-alpha (mRNA-Prkaca). GABRA1 is the only membrane protein [39]. It can activate gamma-aminobutyric acid (GABA) receptor and inhibit receptor-mediated Ca^{2+} influx [40], which is related to the efficacy of *Angelica dahurica*. So GABRA1 was deduced as potential target for the active compounds of *Angelica dahurica*.

The three active compounds had same parent nucleus (psoralen), so they may all interact with the potential target (GABRA1). After the structures of GABRA1 (ESM Fig. S8) and the three active compounds were input into docking software system Dock in turn, the binding portfolio of compound-protein pairs was obtained. The interaction between active compounds and target (GABRA1) is shown in Fig. 8b–d and ESM Fig. S9. In addition, native ligand and thiourea were also evaluated by molecular docking as control (Fig. 8a, e). The related binding sites are listed in Table S3 (see ESM). The docking scores for native ligand, imperatorin, pabulenol, bergapten, and thiourea were 6.220, 6.200, 2.849, 2.697, and 5.881, respectively. Though the docking score of thiourea was high, it only represented the strength of binding force, rather than pharmacological effect. In addition, the number of binding sites for thiourea on GABRA1 was only 1, and the binding site for thiourea was different from that for native ligand. On the contrary, the numbers of the same binding sites between active compounds and native ligand were 5 (imperatorin), 2 (pabulenol), and 4 (bergapten), respectively. Therefore, the active compounds could play a similar role of native ligand, resulting in the activation of GABRA1. Bergapten was not recorded by TCMSP, and its research was rare. But bergapten could be fished out by CMCFP, and it was deduced as a new potentially active compound by network construction and molecular docking. These results indicated that the CMCFP can be applied for fishing unknown and active compounds from natural product.

Comparison with fishing of CM

To further evaluate the advantages of using CMCFP for fishing, the CM of RBC was applied for fishing directly according to the previous report [21]. For evaluating the selectivity, the CM of RBC was used to fish from a mixture of verapamil and thiourea (ESM Fig. S6). The washing solution and the eluent from CM after incubation with the mixture or normal saline have some miscellaneous peaks, which were presumed to be components from CMs. On the contrary, no miscellaneous peak was observed in fishing of CMCFP (Fig. 5), which indicated that the immobilization of CM on CFP was beneficial to the stability of CM in fishing process. In addition, the CM was used to fish directly from the extract of *Angelica dahurica* (ESM Fig. S7). The chromatogram of eluent from CM after incubation with extract had many miscellaneous peaks, which disturbed the identification of active compounds (ESM Fig. S7b). These result demonstrated that the fishing assay based on CMCFP was more effective than that on CM, as well as the preparation of CMCFP was simpler and cheaper than other strategies [3, 7, 13, 17, 22].

Conclusion

In this paper, the CMCFP was for the first time fabricated and used for ligand fishing assay. The confocal imaging demonstrated that the CM was immobilized onto the CFP by the insertion and self-fusion process. The CMCFP was also applied for fishing from the extract of *Angelica dahurica*, and three potential active compounds, including bergapten, pabulenol, and imperatorin, were identified. After retrieving by TCMSP, the GABRA1 was deduced as potential target for the active compounds of *Angelica dahurica*. The interaction between active compounds and GABRA1 was evaluated by molecular docking, and bergapten was deduced as a new potential active compound. Furthermore, the preparation process of CMCFP and fishing assay based on CMCFP were more effective, simpler, and cheaper than other method. Therefore, the proposed CMCFP can serve as an alternative fishing material for screening active compounds from natural product. In

our group, the immobilization of receptor-high-expression cell (e.g., cancer cell) is being carried out for screening active compounds from TCM.

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Compliance with ethical standards

The procedure about rabbit was approved by Animal Ethics Committee, Tianjin Medical University, Tianjin, China. All the experiments on rabbits were performed in compliance with the guide of care and use of laboratory animals.

Conflict of interest The authors declare that they no conflict of interest.

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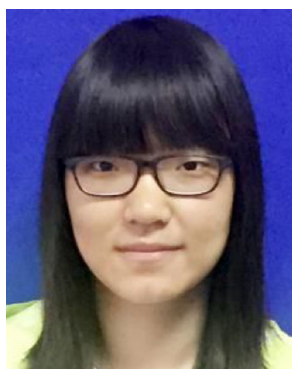
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