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Screening and identification of ligand-protein interactions using functionalized heat shock protein 90-fluorescent mesoporous silica-InP/ZnS quantum dot nanocomposites

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ABSTRACT

Currently, nanosphere-based ligand fishing cannot be accomplished with imaging processing, although this step is important for real-time identification. Herein, a ligand fishing technique combined with *real-time* imaging is presented for the identification of ligands for heat shock protein 90α (Hsp 90α) from a complex matrix, *Alisma plantago-aquatica Linn*. crude extract, using Hsp 90α-functionalized mesoporous silica nanoparticle (MSN)-InP/ZnS quantum dot (QD) nanocomposites as a support material. Twenty ligands for Hsp 90α were screened, and their structures were identified by mass spectrometry. The activities of the ligands were verified by *real-time* imaging of cells apoptotic morphological changes. Quantitative analysis showed that *Alisma plantago-aquatica Linn* contained 8.19 μg/g Alisol F, which regarded as one typical component of *Alisma plantago-aquatica Linn*, and the extraction ratio of Alisol F was 76.2%. The precision for five replicate measurements was 7.0% (RSD). The prepared nanocomposites were also used to screen proteins from a mixture of cellular extracts, and five proteins from HeLa cells were identified as potential client proteins of Hsp 90α.

1. Introduction

Heat shock protein 90 (Hsp 90α) is a kind of complex chaperone machinery in the eukaryotic cells [1]. Hsp 90α and its co-chaperones can guide the correct folding of cellular proteins, and which are also regarded as effectors of signal transduction pathways, controlling cell growth, DNA damage response and cell survival [2,3]. Hsp 90α has already been considered as a cancer target protein in recent years because it participates in key processes in oncogenesis [4]. Additionally, many client proteins of Hsp 90α are also responsible for the development and progression of cancer [5]. The inhibition of Hsp 90α activity can lead to the simultaneous degradation of Hsp 90α and the oncogenic client proteins; thus, searching for Hsp 90α inhibitors and identifying potential client proteins is very important for anti-cancer drug development [6].

To date, although many Hsp 90α client proteins have been identified and some of them play important roles in oncogenic development and cancer cell survival, there still may be many unidentified client proteins that could help clarify new carcinogenicity-related discoveries. Co-immunoprecipitation is still a primary method utilized for screening client proteins. However, it cannot be applied to finding new proteins, especially without first knowing what proteins are being searched for. Moreover, potential nonspecific binding interactions and cross-contamination are becoming additional difficult problems to solve [7–9]. Screening techniques based upon the binding specificity between proteins and biomolecules have been proven to be a convenient and powerful approach to screening for potential client proteins [10,11].

On the other hand, searching for ligands from herbal plants that can inhibit Hsp 90α has attracted special attention in anti-cancer research because a variety of Hsp 90α inhibitors, such as 17-allylamino-17-demethoxygeldanamycin (17-AAG) and epigallocatechin gallate (EGCG), were discovered from natural products [12,13]. *Alisma plantago-aquatica Linn*., a rhizomatous herbaceous perennial plant of the Alisma orientalis (Sam.) Juzep family, com-

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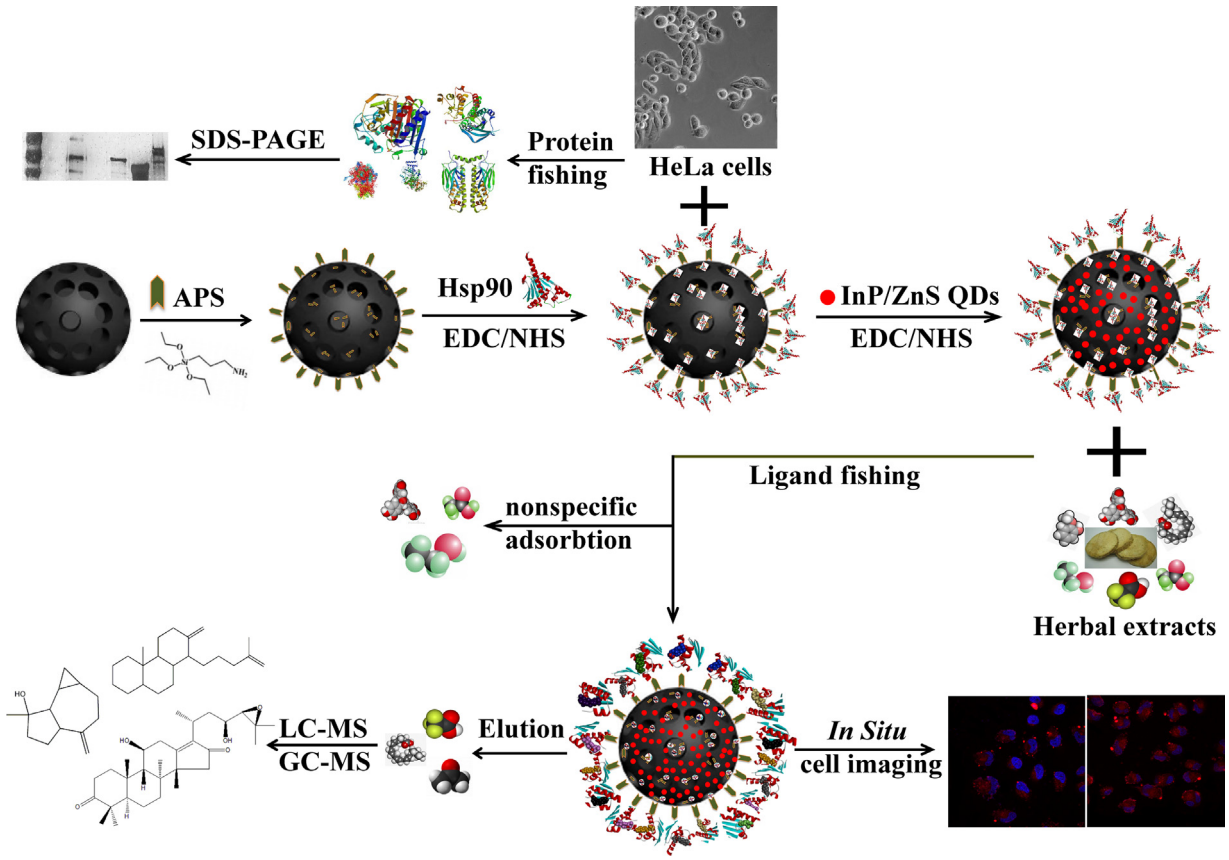

Fig. 1. Schematic illustration of Hsp 90α-coated fluorescent mesoporous silica-InP/ZnS QD nanocomposites and their application in screening client proteins from HeLa cells and small ligands of Hsp 90α from a complex matrix, *Alisma plantago-aquatica Linn.* crude extract.

monly serves as a traditional herbal medicine for the adjuvant treatment of cancer [14], hyperlipemia, diabetes [15] and many other diseases [16,17] in China, Japan, and India [18–20]. However, the direct screening and identification of potential bioactive molecules by gradual separation from *Alisma plantago-aquatica Linn.* is generally difficult due to its extremely complex composition as well as low contents of the bioactive molecules (often less than 1%).

At present, the most widely utilized high-throughput screening (HTS) methods based on colorimetric or fluorometric assays on multiwell microplates are unsuitable for the screening of complex samples such as herbal extracts, although HTS works well with large libraries of synthetic compounds [21]. In recent years, ligand fishing has been proved to be a very effective approach to discovering potential biologically active compounds [22,23,30]. However, a limitation of this method is that to date, it does not allow biomedical imaging that further shows *in situ* screening results.

Semiconductor quantum dots (QDs) have shown great potential in biolabeling and bioimaging due to their exceptional optical properties, including their size-tunable and narrow emission, wide absorption and high photostability [24–26].

Herein, we demonstrate an effective approach to screening and identifying small-molecule ligands of Hsp 90α from a complex natural product, *Alisma plantago-aquatica Linn*, and client proteins present in a complex matrix based on affinity extraction using target protein-mesoporous silica nanoparticle-quantum dot (MSN-QD) conjugates, as shown in Fig. 1. The small-molecule ligands screened from the *Alisma plantago-aquatica Linn.* matrix were preliminary identified by ultra-flow liquid chromatography coupled with quadrupole-time-of-flight mass-spectrometry (UFLC/Q-TOF-MS), gas chromatography-mass spectrometry (GC–MS) and molecular docking. The potential biological activity on cancer cells was verified by *real-time* cell imaging. The screened proteins were identified by nano LC-ESI–MS and gel electrophoresis.

2. Experimental section

2.1. Chemicals and materials

3-Aminopropyltriethoxysilane (APS) and hexadecyl trimethyl ammonium bromide (CTAB) were purchased from Shanghai J&K China Chemical Ltd. InP/ZnS QDs (10 nmol InP/ZnS_MPA QDs, PL = 646 nm, FWHM = 75 nm, QY ~20%) were obtained from Mesolight Inc. Tetraethyl orthosilicate (TEOS) and mesitylene were purchased from Aladdin Industrial Corporation. Potassium chloride (KCl), N-hydroxysuccinimide (NHS), sodium hydroxide (NaOH), sodium chloride (NaCl), ammonium nitrate (AN), chlorine hydride (HCl), potassium phosphate monobasic (KH_2PO_4), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$), 1,2-dichloroethane, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and tri (hydroxymethyl) amino methane hydrochloride (Tris-HCl) were purchased from Sangon Biotech Co., Ltd.

2.2. Plant materials

Dried *Alisma plantago-aquatica Linn.* was obtained in January 2016 from Beijing Tong Ren Tang (BoZhou) Herbal company. A voucher specimen was deposited in the School of Pharmacy, Nanjing University of Chinese Medicine, China.

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