Rapid naked-eye tracking of on-cell phenotype based on dual-aptamers-weaved cascade assembly of nanostructures

Genxi Li (genxili@nju.edu.cn)
Nanjing University

Article

Keywords:

Posted Date: March 23rd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-269749/v1

Abstract

Phenotypic plasticity is an emerging paradigm for providing biological and clinical insights into cancer initiation, progression, and resistance to therapy. However, it is a great challenge to tracking phenotypic information on live cells with high levels of sensitivity, specificity, and simplicity, when specific cancer cell subset is being targeted. In this work, we have successfully achieved cascade assembly of nanoparticles on the surface of specific cancer cells by design of a dual-aptamers-weaved molecularly AND logic system. Taking advantages of spatial addressability, precise controllability, and targeting recognition of the nanostructure assemblies, we can precisely label the target cell subset in large population of similar cells and rapidly obtain phenotypic information in response to the surface changes of captured cancer cells. Without sophisticated instruments, we can know the phenotypic information on HepG2 cells in whole blood with high level of sensitivity, and rapid naked-eye tracking of on-cell phenotype changes of HepG2 cells undergoing epithelial-mesenchymal transition (EMT).

Introduction

Phenotypic plasticity is directly related with the cellular origins of tumor, tumor progression and metastasis, and resistance to therapy[1]. Therefore, to better understand the roles of heterogeneous phenotypes in cancer cells, it is highly required to develop non-invasive and non-destructive method that can efficiently isolate specific sub-population of cancer cells from a bulk population, accurately extract phenotypic information, and timely track their phenotype changes. Considering that the presence or absence, abundance or scarcity of certain surface proteins can be the indicator of more aggressive cancer sub-populations, which shows the characterization of disseminated tumor cells (DTCs)[1-3], cell surface proteins-based cell phenotype analysis may contribute to understanding how the phenotype affects tumor progression. Compared with intracellular markers such as nuclei acids and small molecules[4, 5], cell surface proteins are excellent targets for cell separation and characterization due to their accessibility and ease of targeting *via* specific antibody/aptamer recognition[6-8].

At present, conjoint analysis of multiple cell surface proteins is recommended in the profiling of cell phenotype[9-11]. This is because single surface protein-based analytical methods easily produce “false positive” or “false negative” results, resulting in the lack of indicators of early warning, and unsatisfying the accurate analysis of low abundance of cancer cells in the early stage of tumor development[12]. Nevertheless, conjoint analysis of multiple cell surface proteins is still challenging due to some existed issues. First, selecting cell surface proteins for conjoint analysis is remarkably difficult, because most cell surface proteins can be detected on various cancer cell lines, which are lack of sufficient specificity. Some existed methods can narrow down a small population of cells from a sea of cells, but often fail to detect more aggressive subpopulations. Another issue is the limitation of analysis techniques. So far, fluorescence readout-based microfluidics and flow cytometry are main techniques for the profiling of cancer cells[13-16]. However, flow cytometry requires expensive sophisticated equipment and skilled technical personnel. Microfluidics-based techniques require preparation of a special device without unified standards. Moreover, the existing methods are generally limited to the capture and enumeration of cancer cells and do not provide the phenotypic information of cancer cells. In recent years, electrochemical or spectroscopic approaches have been proposed for the profiling of cell phenotype[17, 18]; however, they often have trade-offs in performance metrics such as sensitivity, specificity, and simplification. Therefore, there is an ever-increasing need for gentle and scalable method to sort and characterize cancer cells according to their detailed phenotypic profiles, so that the properties of invasive versus noninvasive cells can be identified.

To meet these challenges, we propose a practical method based on dual-aptamers-weaved cascade assembly of nanostructures in this work. To focus on a specific cell subpopulation, a cell-specific aptamer is firstly used for fishing target cell from a bulk population. Cell aptamer is generated through cell-based systematic evolution of ligands by exponential enrichment (cell-SELEX), it can specifically bind to a certain cell line based on unique extracellular characteristics, while no prior knowledge of cell surface proteins is required[19, 20]. Subsequently, a cell surface protein-specific aptamer is used to combine with the cell-specific aptamer to form a molecularly AND logic device, thus cascade assemblies of two functionalized nanoparticles with high catalytic activity can be triggered on live cancer cells. Taking advantages of spatial addressability, precise controllability, and targeting recognition of the nanostructure assemblies, we sought to resolve the challenges with the rational design and successful operation of multiple aptamers-mediated nanoparticles assemblies for accurate cancer cell subpopulation fishing in cell mixtures and rapid naked-eye tracking of on-cell phenotype in epithelial-mesenchymal transition (EMT).

Results And Discussion

Schematic diagram of the system design and operational mechanism

First of all, to acquire profiles of specific cancer cell subpopulation, cell-specific aptamer and cell surface protein aptamer are used as two engaged cogwheels for forming a molecularly AND logic device to narrow down a specific cancer cell subtype from a sea of cells (**Scheme 1**). This AND logic device is composed of triple

‘lock-and-key’-controlled system, which ensures high specificity and sensitivity for cancer cells identification and profiling by their sequential activation. The first ‘lock-and-key’ system is consisted of cell-specific aptamer and hairpin DNA (HP-1)-conjugated SA@Fe_3O_4 beads (HP-1-SA@ Fe_3O_4), which enables to fish cell aptamer-targeted subpopulations from a sea of cells and is responsible for the activation of second ‘lock-and-key’ system after hybridization reaction of cell-specific aptamer with HP-1-SA@Fe_3O_4. The above hybridization reaction releases the accessible nicking site of restriction endonuclease Nb.BbvCI, activating the second ‘lock-and-key’ system containing Nb.BbvCI and hairpin DNA (HP-2) functionalized platinum-coated gold nanoparticles (Pt@AuNPs) (Pt-1). Upon addition of Nb.BbvCI, intact cleavage site formed by the loop of HP-2 and released DNA sequence can be cleaved to produce single-stranded DNA (ssDNA)-linked Pt-1 (ssDNA-Pt-1). The cleaved ssDNA-Pt-1 can further initiate downstream third ‘lock-and-key’ system consisted of epithelial cell adhesion molecule (EpCAM) aptamer and hairpin DNA (HP-3) modified platinum-coated gold nanoparticles (Pt-2). If the signature EpCAM is expressed on cancer cells, ssDNA-Pt-1 can assemble on the cancer cell surface by hybridizing with the extended non-aptamer hairpin DNA (same sequence with HP-3) of EpCAM aptamer, while the generated ssDNAs on Pt-1 simultaneously hybridize with HP-3 on Pt-2, resulting in the layer-by-layer assembly of Pt-1 and Pt-2 to form 3-

dimensional nanoparticles complex for signal integration and amplification. All components in the 'triple lock-and-key'-controlled system are operationally connected and can be cascaded autonomously by dual-aptamers-based molecularly AND logic system. Besides, taking advantage of the excellent catalytic activity of platinum-coated gold nanoparticles[21, 22], highly sensitive colorimetric assay of cancer cells can be achieved, thus naked-eye profiling of cell phenotype can be carried out by making use of the reaction of 1, 3, 5-trimethylbenzene (TMB) with hydrogen peroxide (H_2O_2).

Feasibility of Nb.BbvCI-induced DNA assembly

Nb.BbvCI cleavage reaction-induced DNA assembly is a bridge between these 'lock-and-key'-controlled systems, thus a fluorescence experiment has been designed and performed for verifying its feasibility (Supplementary Fig. 1). An ssDNA (ssDNA1) is firstly used as the substitute of the released recognition sequence of Nb.BbvCI induced by the hybridization of cell aptamer and HP-1-SA@Fe_3O_4, which can hybridize with FAM/BHQ1-labeled HP-2 to form intact cleavage site of Nb.BbvCI (Supplementary Fig. 1A). After cleavage reaction, obvious fluorescence increase can be detected, which shows that HP-2 is cleaved by Nb.BbvCI to generate a free FAM-labeled ssDNA, resulting in the fluorescence recovery (Supplementary Fig. 1B, C). Next, the extended non-aptamer hairpin DNA of cell surface protein aptamer (i.e., HP-3) is added into the assembly reaction solution, and a further fluorescence increase can be measured by a fluorescence spectrophotometer. The result clearly demonstrates that the cleaved ssDNA can further open hairpin structure of HP-3, promoting disassembly of the cleaved HP-2 and accompanied fluorescence enhancement. As a control, no obvious fluorescence increase is observed in the absence of Nb.BbvCI or ssDNA1. Therefore, these results clearly verify the feasibility of Nb.BbvCI-induced DNA assembly.

Characterization of the functionalized DNA nanoprobes

Multifunctional HP-2 and HP-3 are linked on the synthesized Pt@AuNPs with high catalytic activity to prepare nanocatalyst amplification platforms (Pt-1 and Pt-2), respectively (Supplementary Fig. 2A). They are responsible for the subsequent signal integration and amplification. We have used several techniques to investigate the functionalized Pt@AuNPs, confirming the successful preparation of two nanocatalyst amplification platforms. Firstly, dynamic light scattering has been performed to analyze the hydrodynamic diameter changes in the functionalization process of Pt-1 (Supplementary Fig. 2B). The results show that the hydrodynamic diameter of Pt@AuNPs increases from 42.97 nm to 44.71 nm compared with that of AuNPs, and further increases to 75.25 nm after loading HP-2. Then transmission electron microscopy (TEM) images characterize that the average diameter of Pt-1 is ~20 nm, and they present good dispersibility (Supplementary Fig. 2C). The fabrication of Pt-1 is further confirmed from UV–vis spectrometric results, in which a red shift of the absorption peak of AuNPs appears after cladding multiple layers of Pt atom and loading HP-2 (Supplementary Fig. 2D). Besides, the peroxidase-like catalytic property of Pt-1 is investigated with the oxidation of TMB by H_2O_2, and the kinetic assay results show that Pt@AuNPs have higher catalytic efficiency than the initial AuNPs (Supplementary Fig. 2E). From the above, all characterization results suggest that functionalized Pt@AuNPs with high catalytic efficiency have been successfully prepared.

Dual-aptamers-based AND logic system for cancer cell identification.

To clearly demonstrate whether our AND logic system can work for accurate cell identification and profiling, we have designed a model by employing cell-specific aptamer sequence TLS11a and EpCAM-targeting aptamer sequence, against EpCAM-positive human hepatocellular carcinoma cell line HepG2. Then, a competition experiment has been conducted to verify that cell aptamer and EpCAM aptamer can bind to different sites on HepG2 cells, while no obvious interference is observed when the two aptamers are incubated together with cancer cells (Supplementary Fig. 3). Next, we need to know if efficient nanoparticles assemblies on cancer cells can be obtained by this design of the triple 'lock-and-key'-controlled system. For this purpose, we have dissected each key component in the AND logic system and tested the effects of their lack on whole assembly reaction, including Pt-1, Pt-2, EpCAM aptamer, Nb.BbvCI, and target cell (Fig. 1A). The UV–vis spectrometric results and visual color changes generated through the catalytic reaction have been separately recorded (Fig. 1B, C). First, we perform the intact operation in the presence of all components, and a dark blue-colored product (i.e., oxTMB) can be generated after adding TMB and H_2O_2. As a control, when HepG2 cells are not added into the reaction, only light blue is observed and much lower absorbance is detected. The results can be attributed to failing to obtain a large number of nanoparticles assembly products on the surface of HepG2 cells. For the detected background signal, it may be generated by the nonspecific adsorption between SA@Fe_3O_4 and nanocatalysts. Similarly, in the absence of Pt-1, or Pt-1/Pt-2, the reaction solution is also light blue and similar lower absorption peaks are measured. It should be noted especially that obvious color change can be visualized in the absence of Pt-2, because Pt-1 can still be assembled on cancer cells after cleavage reaction of Nb.BbvCI. However, in comparison, 2.28-fold absorption value increase can be measured in the presence of Pt-1 and Pt-2, verifying the introduction of Pt-2 can contribute to excellent signal amplification. Further experiments demonstrate that the absence of cell aptamer or EpCAM aptamer can also inhibit the assembly of nanostructures on cancer cells, suggesting that cell-specific aptamer and cell surface protein aptamer can

be used as two engaged cogwheels for forming a molecularly AND logic device. In summary, these results clearly verify our dual-aptamers-based AND logic device can work for accurate cell identification, which will help to further profile in depth.

Optimization of several key experimental conditions

We have investigated the effects of several key experimental conditions on the assay performance of our AND logic system, including non-specific adsorption, nanocatalyst concentration, nanoparticles assembly time. First, a high background signal produced by the non-specific adsorption between SA@Fe_3O_4 and Pt@AuNPs is observed in the experimental process. To obtain an optimal signal to noise ratio (SNR), a block strategy has been carried out with BSA and thiol-

labeled poly(T) DNA (Supplementary Fig. 4). Results indicate that the non-specific adsorption between SA@Fe_3O_4 and Pt@AuNPs can be remarkably reduced by incubating BSA and thiol-labeled poly(T) DNA with Pt@AuNPs, so the block strategy continues to be used in the following experiments. Next, we have optimized the concentrations of Pt-1 and Pt-2 (Supplementary Fig. 5). The optimal Pt-1 concentration is evaluated in the absence of Pt-2. The results indicate that the absorption intensity at 450 nm increases gradually with the elevated concentrations of Pt-1 and tends to equilibrium until the concentration of Pt-1 is 64 μg/mL, which are also confirmed by the visual color changes of reaction solution (Supplementary Fig. 5A). It is noteworthy that the background signal generated from non-specific interaction between HepG2 cells and Pt-1 in the absence of EpCAM aptamer can also be observed. It increases with the Pt-1 concentration and the absorption maximum is obtained at 64 μg/mL of Pt-1. By analyzing the ratio of UV–vis absorption intensities at 450 nm in the presence/absence of EpCAM aptamer, an acceptable SNR can be obtained with 64 μg/mL of Pt-1. The concentration of Pt-2 is evaluated in following experiments (Supplementary Fig. 5B). Similarly, it can be observed that the absorption intensity at 450 nm increase gradually with the elevated concentrations of Pt-2 when Pt-1 concentration is fixed. It tends to equilibrium until the concentration of Pt-2 is 64 μg/mL. These results also demonstrate that Pt-1 and Pt-2 can be sequentially assembled on cancer cells induced by dual-aptamers-medicated AND logic system for signal integration and amplification. Finally, we have investigated the reaction time of whole assembly process. The results show that the whole assembly process is rapid, only 30 min is needed for the operation of dual-aptamers-medicated AND logic system (Supplementary Fig. 6).

Application of the logic system for quantification detection of HepG2 cells

Under optimized conditions, we have examined the application of this proposed AND logic system for cancer cells analysis. To characterize the analysis performance, the multilayered assembly of nanoparticles is operated on different concentrations of live cancer cells (Fig. 2). The photographs of the oxidation products of TMB by H_2O_2 are

plotted in Fig. 2A after the addition of HepG2 cells ranging from 0 to 1.0×10^4 cells/mL. It can be visualized that the color of reaction solution gradually changes from light blue to dark blue with the increasing amount of target cells. After the concentration of target cells rises to be 2.0×10^3 cells/mL, the observed changes in color are almost the same. In addition to visual analysis, the performance of this system has been further verified by UV–vis absorption spectra (Fig. 2B). The intensity of the absorption peak at 450 nm gradually increases with the increase of cell concentration. Moreover, the absorption intensity and cell concentration have a positive linear correlation with a linear response range from 10 to 1000 cells/mL, and the detection limit is < 10 cells/mL. To further verify nanoparticles assembly-dependent changes in color, we incubate TMB and H_2O_2 with cell lysate of different concentrations of HepG2 cells (Supplementary Fig. 7). The results clearly demonstrate that obvious color changes are visualized in the series of samples (inset in Supplementary Fig. 7), suggesting the color changes are not generated from the interaction between TMB and H_2O_2 catalyzed by cell lysate, which further verifies the feasibility of our AND logic system.

We have also investigated the programmability of this logic system with a re-designed system weaved by HepG2 cell-specific aptamer and E-cadherin-targeted aptamer (Supplementary Fig. 8). Subsequently, the application of this proposed AND logic system for cancer cells analysis is tested by performing Pt@AuNPs assemblies on cancer cells. The recorded photographs of color changes and measured absorption intensities can verify that the reprogrammed system can also be used for cancer cells sorting and analysis. However, it can be observed that the assembly efficiency of the reprogrammed system is significantly lower than the previous system, which may be attributed to the lower affinity of E-cadherin aptamer to E-cadherin than that of EpCAM aptamer to EpCAM. Meanwhile, the two molecularly AND logic systems confirm the feasibility of our design of dual-aptamer-mediated cascade nanoparticles assembly.

Control experiments have been performed to test the selectivity of this logic system. Firstly, we carry out the AND logic operation on different cancer cell lines, including hepatocellular carcinoma cell line MHCC97-L, human breast cancer cell lines MCF-7 and MDA-MB-231, and human fetal hepatocyte line L-02 (Fig. 3). The UV–vis absorption spectra show that a much higher absorption intensity can be detected when the AND logic system is operated on HepG2 cells than those of other cell lines (Fig. 3A). The photographs of the oxidation products of TMB by H_2O_2 are inserted in Fig. 3B, which further verifies the results. Secondly, these cell lines are mixed to incubate with all components. Experimental results reveal that our system can still fish HepG2 cells from mixed cells for further colorimetric analysis. To test whether the as-proposed method enables for analyzing cancer cells in a complex environment, a standard addition method has been performed by adding different concentrations of HepG2 cells into 25% human whole blood and 100% undiluted

human serum. As a result, the UV–vis absorption intensities at 450 nm are measured to be proportional to HepG2 cells concentration (Fig. 3C) and obvious color changes are visualized with increasing HepG2 cells concentration (Fig. 3D), demonstrating that this proposed method can detect cancer cells in biological samples.

Application of the system for rapid naked-eye tracking of on-cell phenotype

As is well known, changes in cell phenotype are highly dynamic and heterogeneous in time and space, which plays a vital role in determining oncogenesis, tumor progression, and tumor resistance[23-25]. Thus, a gentle and scalable method is highly needed to sort and characterize cancer cells according to their detailed phenotypic profiles, so that the properties of invasive versus noninvasive cells can be identified. As previously mentioned, we have successfully performed cascade assembly of nanoparticles on live cancer cells by dual-aptamers-mediated AND logic system, which enables us to precisely fish a small collections of target cells in a bulk population and the colorimetric readout has been validated to be response to the change of cell surface phenotype, showing the potential capability to track on-cell phenotype.

To investigate the performance of our proposed method in tracking on-cell phenotype, transforming growth factor-β (TGF-β) is used for inducing epithelial-mesenchymal transition (EMT) of HepG2 cells[26-28], and the dual-aptamers-mediated nanoparticles assembly are performed for tracking cell phenotype changes in EMT process (Fig. 4A). It has been reported that cell phenotype is highly dynamic and heterogeneous in EMT process[29, 30]. As a result, when different concentrations of TGF-β are used for treating HepG2 cells for 24 h and 72 h, respectively, we can observe obvious color changes along with increasing TGF-β concentrations (Fig. 4A). Meanwhile, decreased UV–vis absorption intensities at 450 nm can also be detected with increasing TGF-β concentrations (Fig. 4B). Fig. 4C shows the representative morphology changes in EMT process. The morphology of HepG2 cells gradually changes from the cobblestone-like epithelial appearance to an elongated, spindle-like fibroblastic shape after adding different concentrations of TGF-β. These results clearly indicate that our as-proposed dual-aptamers-mediated AND logic system can be used for rapid naked-eye tracking of on-cell phenotype. The observed color changes and gradually decreased UV–vis absorption intensities may be attributed to the decreasing affinity between HepG2 cells and two aptamers in EMT process. Changes of cell phenotype induced by EMT may result in the loss of cell capture capability of the cell aptamer, and the reduced expression of EpCAM in EMT process[31, 32] can also lower the affinity between EpCAM and aptamer (Fig. 4D).

Conclusion

By using a cell aptamer and a cell surface protein aptamer as two engaged cogwheels for forming a molecularly AND logic device, we have successfully fished a specific cancer cell subtype from a sea of cells and achieved the accurate profiling of on-cell phenotype. The AND logic device is consisted of triple 'lock-and-key'-controlled system, in which all components are operationally connected and can be cascaded autonomously, resulting in multilayered assembly of nanoparticles on the surface of specific cancer cells. Taking advantages of spatial addressability, precise controllability, and targeting recognition of the nanostructure assemblies, we have achieved highly sensitive colorimetric detection of cancer cells (LOD < 10 cells/mL) and naked-eye tracking of on-cell phenotype changes in TGF-β-induced EMT of HepG2 cells. The strategy of dual-aptamers-weaved cascade assembly of nanostructures may provide a generalizable approach to perform accurate on-cell phenotype profiling of various cancer cells of interest by using high affinity aptamers.

Methods

Reagents and materials. Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.99% purity) and Sodium hexachloroplatinate (IV) hexahydrate ($Na_2PtCl_6 \cdot 6H_2O$, 98% purity) were purchased from Alfa Aesar Co., Inc. (MA, USA). Streptavidin (SA), bovine serum albumin (BSA), sodium citrate dehydrate (≥99% purity), L-ascorbic acid (≥99% purity), potassium chloride (KCl), Sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$), Sodium phosphate dibasic ($NaHPO_4$), and sodium chloride (NaCl) were obtained from Sigma-Aldrich (Shanghai) Trading Co., Ltd (Shanghai, China). TGF-β1 was obtained from Sino Biological Inc. (Beijing, China). 0.3 μm streptavidin magnetic bead ($SA@Fe_3O_4$) was purchased from Beaver Biomedical Engineering Co., Ltd. (Suzhou, China). Ribonucleic acid (yeast) was provided by Solarbio Bio-Technology Co., Ltd. 1, 3, 5-trimethylbenzene (TMB) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Hydrogen peroxide (H_2O_2) was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Fetal bovine serum (FBS) was obtained from Thermo Fisher Scientific, Inc. (MA, USA). Dulbecco's modified Eagle medium (DMEM, high glucose) was provided by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). The synthesis and modification of all used DNA sequences were performed by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). All used chemical reagents in this work were analytical grade without further purification. Ultrapure water with a resistivity of 18.2 MΩ·cm was purified by a Milli-Q apparatus.

Synthesis of gold nanoparticles (AuNPs) seeds. AuNPs with a mean diameter of 20 nm were synthesized by a seed-mediated growth approach according to previous work[33]. In brief, sodium citrate solution (2.2 mM, 40 mL) was heated to boiling in a three-neck flask with round bottom for 15 min under vigorous stirring. Then $HAuCl_4$ solution (25 mM, 267 μL) was added and allowed to react until the color of the reaction solution changed from yellow to soft pink (~10 min). The synthesized Au seeds were cooled to 90 °C, and then sodium citrate solution (60 mM, 267 μL) and $HAuCl_4$ solution (25 mM, 267 μL) were injected sequentially with 2 min of time delay. Next, 20 nm AuNPs were obtained through repeating the above operation (namely, sequential addition of sodium citrate and $HAuCl_4$ solution). Finally, the prepared AuNPs were required to cool to room temperature and store at 4 °C before use.

Synthesis of platinum-coated gold nanoparticles (Pt@AuNPs). Pt@AuNPs was synthesized according to previous reported method with a minor modification[21]. In a standard synthesis of Pt@AuNPs, prepared AuNPs seeds (8 mL) and 3653 μL ultrapure water were injected in a 50 mL three-neck flask with round bottom. The solution was preheated to 90 °C under magnetic stirring for 10 min, then Na_2PtCl_6 solution (1.0 mM, 347 μL) and L-ascorbic acid solution were added separately into three-neck flask at rates of 1.2 and 2.4 mL/h by using a syringe pump. After complete injection, the reaction was allowed to proceed for an additional 30 min. Finally, the synthesized Pt@AuNPs were cooled down to room temperature, and stored in dark at room temperature before use.

Preparation of DNA-functionalized Pt@AuNPs. To link DNA on Pt@AuNPs, 10 mL prepared Pt@AuNPs solution was firstly adjusted to pH 9.0 using Na_2CO_3 solution under magnetic stirring. Then, streptavidin (SA) solution (1mg/mL, 30 μL) was incubated with the Pt@AuNPs suspension overnight at 4 °C. The following day, BSA solution (10%, 1 mL) was added into the reaction solution. After 1 h, the obtained products were collected by centrifugation and washed thrice with PBS (2 mM NaH_2PO_4, 10 mM $NaHPO_4$, 0.2 M NaCl, 0.1% Tween 20, pH 7.4). Finally, the produced SA-coated Pt@AuNPs were redispersed in 1 mL PBS for further use.

DNA-functionalized Pt@AuNPs were prepared by incubating 150 μL 10 μM biotin-labeled HP-2 or HP-3 with 1 mL SA-coated Pt@AuNPs suspension for 1 h at 37 °C under 800 rpm oscillation. Then, further block was performed by using 1 μM thiol-labeled poly(T) DNA and 1% BSA. Finally, the reaction solution was washed thrice with PBS and the obtained DNA-functionalized Pt@AuNPs were redispersed in PBS.

Characterization of DNA-functionalized Pt@AuNPs. The morphologies of the synthesized AuNPs, Pt@AuNPs, and DNA-functionalized Pt@AuNPs were characterized by a Tecnai G2 F20 S-TWIN transmission electron microscopic (FEI, USA). The particle sizes of all nanoparticles were acquired via dynamic light scattering on a Malvern Zetasizer Nano-ZSE system (Malvern Instruments Ltd, UK). The ultraviolet−visible (UV−vis) spectra were performed using a Shimadzu UV-1800 spectrometer (Shimadzu Inc., Kyoto, Japan).

Preparation of functionalized magnetic Fe_3O_4 beads. 0.3 μm SA@Fe_3O_4 (1 mg/mL, 500 μL) and HP-1 (10 μM, 75 μL) were mixed and incubated for 1 h at 37 °C under 800 rpm oscillation. Then, HP-1-functionalized SA@Fe_3O_4 was washed with PBS for three times and magnetic separation was performed to collect the products.

Cell culture. Human hepatocellular carcinoma (HCC) HepG2 cells, HCC MHCC97L cells, human cervical carcinoma HeLa cells, human breast carcinoma MDA-MB-231 cells, and human breast carcinoma MCF-7 cells were cultured in DMEM containing 10% FBS, penicillin (100 μg/mL), and streptomycin (100 μg/mL). Human normal liver L-02 cells were cultivated in Roswell Park Memorial Institute 1640 medium (RPMI 1640, Gibco) added with 10% FBS, penicillin (100 μg/mL), and streptomycin (100 μg/mL). All cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2.

Epithelial-mesenchymal transition (EMT) induced by TGF-β. To induce EMT of HepG2 cells, different concentrations of TGF-β were incubated with HepG2 cells in DMEM for 24 h and 72 h, respectively. These treated cells were washed with PBS for three times, which were then gently scraped into a sterile tube with cell scraper for colorimetric analysis and flow cytometric assay. For observing morphological change of HepG2 cells in EMT, cells were cultured in 6-well plate and treated with different concentrations of TGF-β.

Colorimetric profiling of cancer cells. All cells were collected by using a cell scraper after being washed twice with PBS (8.1 mM Na_2HPO_4, 1.76 mM KH_2PO_4, 136.89 mM NaCl, 2.67 mM KCl), and dispersed in PBS. For cancer cells detection, different concentrations of HepG2 cells were firstly incubated with the cell aptamer (2 μL 20 μM), EpCAM aptamer (2 μL 20 μM), and HP-1-functionalized SA@Fe_3O_4 (3 μL 5 mg/mL) in PBS for 30 min at 37 °C. Then, 2 μL of yeast tRNA was added and allowed to be proceed for an additional 30 min. Then, cells were washed with PBS for three times and magnetic separation was performed to collect the products. Nanoparticles assemblies were carried out by incubating the separated cells with 40 μL of Pt-1, 40 μL of Pt-2, 1 μL of Nb.BbvCI, and 19 μL of PBS at 37 °C. After 30 min. unassembled nanoparticles were removed via magnetic separation and the produced complex was washed twice with PBS, subsequently, colorimetric readout was obtained by incubating with TMB (0.5 mg/mL) and H_2O_2 (5 mM) for a few minutes in reaction buffer (10 mM $NaHPO_4$, 2 mM NaH_2PO_4, 135 mM NaCl, 4.7 mM KCl, pH = 4.0).

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Declarations

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant Nos. 81772593, 31901771, 81503463) and the Postgraduate Research & Practice Innovation Program of Jiangsu Province (No. SJCX20_0005).

Author contributions

H. S. and G. L. conceived the project. H. S., M. W. and Y. G. designed and performed most of the experiments. H. S., M. W. and Y. H. synthesized Pt@AuNPs. H. S., M. W., L. M., Y. Y. and G. L. wrote the paper. All authors reviewed and modified the manuscript.

Conflicts of interest

The authors declare no competing financial interest.

Additional information

Supplementary information is available for this paper at

Figures

Figure 1

Feasibility test of dual-aptamers-based AND logic system for cancer cell identification. (A) Schematic table of dual-aptamers-based AND logic system for cancer cell identification under different conditions. (B) The corresponding UV–vis absorption spectra in (A). (C) The corresponding visual color changes and UV–vis absorbance intensities at 450 nm.

Figure 2

Operating dual-aptamers-medicated AND logic system for the quantification detection of HepG2 cells. (A) The visual color changes generated from oxidation of TMB and H_2O_2 catalyzed by multilayered assembly of nanoparticles on cancer cells. (B) The UV–vis absorption spectra of TMB oxidation products catalyzed by Pt@AuNPs assemblies on different concentrations of HepG2 cells. (C) The UV–vis absorption peak intensity responses at 450 nm to various concentrations of HepG2 cells. Inset: Correlation curve of the UV–vis absorption peak intensities at 450 nm versus HepG2 cells.

Figure 3

(A-B) The UV–vis absorption spectra and UV–vis absorption intensities at 450 nm of

TMB oxidation products catalyzed by Pt@AuNPs assembled on different cancer cell lines.

Inset in B shows the visual color changes in response to different cancer cell lines.

(C) Correlation curves of the UV–vis absorption peak intensities at 450 nm versus HepG2 cells

in 25% whole blood or 100% serum, respectively. (D) Visual color changes in response to

different concentrations of HepG2 cells in 25% whole blood or 100% serum,
respectively.

Figure 4

(A) Schematic diagram of application of the logic system for naked-eye tracking of on-cell phenotype in EMT process. Inset: Visual color changes in response to different concentrations of TGF-β, HepG2 cells treated with TGF-β for 24 h and 72 h, respectively. (B) Correlation curves of the UV–vis absorption peak intensities at 450 nm versus TGF-β. (C) Representative morphology changes of HepG2 cells in EMT induced by TGF-β. (D) Flow cytometric assay for HepG2 cells treated with different concentrations of TGF-β. 100 nM of cell aptamer or EpCAM aptamer is incubated with 10^5 cells/mL of HepG2 cells at 37 °C after cells are treated with TGF-β for 72 h.

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