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Metastatic cancer cell and tissue-specific fluorescence imaging using a new DNA aptamer developed by Cell-SELEX

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ABSTRACT

Metastasis, which derived from a primary tumor, accounts for 90% of mortality caused by cancer. Early diagnosis of cancer metastasis may significantly improve cure rate of patients who are at high risk for developing metastasis. In this study, we successfully achieved metastatic cancer cell and tissue-specific fluorescence imaging by using a new aptamer developed by cell-based systematic evolution of ligands by exponential enrichment (Cell-SELEX). With metastatic colorectal carcinoma LoVo cells as selection target, the aptamer named J3 which bind to metastatic cancer cells with good affinity and specificity was obtained. Then J3 was labeled with Cy5 fluorescent group (J3-Cy5) for imaging metastatic cancer cells, the results demonstrated excellent imaging contrast. Moreover, the results of tissue section imaging revealed that J3-Cy5 probe explicitly recognized lymph node tissue with colorectal carcinoma metastasis with a high detection rate of 73.9%, but showed a low detection rate to colorectal carcinoma tissue with no metastasis or cancer adjacent tissue. Therefore, the targeting reagent J3-based fluorescence imaging possesses great potential for clinical diagnosis of cancer metastasis.

1. Introduction

Metastasis is one of three known unknowns of cancer, including drug resistance, tumor microenvironment and metastasis [1]. It is responsible for as much as 90% of mortality caused by cancer, yet it remains the most poorly understood content of cancer pathogenesis [2,3]. Although anatomic imaging played a vital role in assessment of cancer metastasis, these conventional methods based on morphology primarily image the advanced metastatic tumors when they are in centimeter scale or greater in diameter [4,5]. However, metastasis may occur in early stage of cancer in terms of circulating tumor cells or microemboli which are difficult to image clearly because of their small amount and size [6]. Molecular imaging possesses great potential to improve diagnosis for metastatic tumor, which depends on interrogating abnormal molecules that appearing in the earliest stage or its aberrant interactions that are the basis of cancer metastasis [7]. Imaging of metastatic tumor at molecular level can be achieved by using specific probes targeted metastasis-related biomarkers. Therefore, imaging probes specific to metastatic tumors are urgently needed to diagnose patients who are at high risk for developing metastasis, which might guide oncologists to design accurate therapeutic strategies for individual patients [8]. Although antibodies are usually used as targeting ligands, aptamers offer several unique advantages over antibodies to be an alternative in molecular imaging [9,10].

Aptamers, also known as chemical antibodies, are short, single-stranded oligonucleotides evolved from an in vitro process called SELEX (systematic evolution of ligands by exponential enrichment) [11–16]. Because of their excellent chemical properties, such as easy synthesis, controllable modifications, long-term stability, low immunogenicity, fast tissue penetration and short blood residence time, aptamers have gained increasing popularity for disease diagnosis and therapy in molecular medicine, especially aptamer-based molecular imaging [17–22]. For instance, an activatable aptamer probe (AAP) targeting acute lymphoblastic leukemia cells was designed by our lab, which achieved enhanced contrast for tumor visualization in living mice because target-induced conformational alteration of the AAP can avoid background signal effectively [23]. Moreover, by applying targeting aptamers with nano-materials, such as graphene oxide [24–26], carbon nanotube [27], silver nanoclusters [28,29] and multi-

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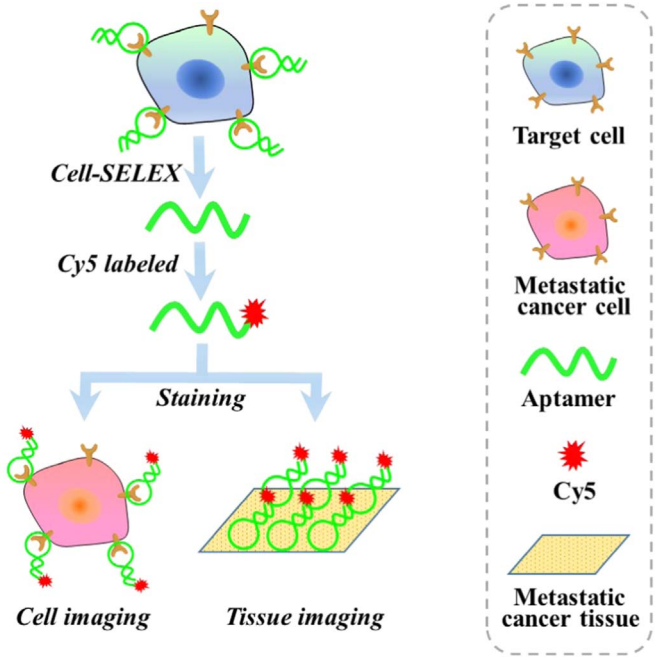
functional nanocomposites [30–32], fluorescence molecular imaging was significantly improved to detect small molecules, proteins and primary cancer cells. In addition, a ^{18}F-labeled aptamer specific to protein tyrosine kinase 7 (PTK-7) was also reported for positron emission tomography (PET) imaging of PTK-7-positive colorectal cancer cells [33]. By modifying the magnetic iron oxide nanoparticles with aptamer as targeting agent, magnetic resonance imaging (MRI) that generally aimed to anatomical imaging could be used for molecular imaging of hepatocellular carcinoma [34]. In another case, the nucleolin aptamer conjugated gold nanoparticles displayed strong X-ray attenuation for computed tomography (CT) contrast enhancement in lung adenocarcinoma-bearing mice [35]. In view of above mentioned performances, aptamer-based methods for imaging metastatic cancer may possess great prospects.

In the present work, colorectal carcinoma, which 50% of diagnosed patients are found with metastasis and the five-year survival rate is only 6% worldwide [36], was chose as a studying object. By using metastatic colorectal carcinoma cell line LoVo as target cell and non-metastatic colorectal carcinoma cell line SW480 as control cell, we have identified a new aptamer J3 which bind to metastatic cancer cells with high affinity and specificity through Cell-SELEX. The proteinase treatment experiments indicated that the target of J3 is mostly like to be metastasis-related membrane protein. When J3 was labeled with Cy5 fluorescent group, J3-Cy5 showed excellent imaging contrast to metastatic cancer cells. Importantly, the results of tissue section imaging revealed that J3-Cy5 explicitly recognized lymph node tissue with colorectal carcinoma metastasis with a high detection rate of 73.9%, but showed a low detection rate to colorectal carcinoma tissue with no metastasis or cancer adjacent tissue (Scheme 1).

2. Experimental

2.1. Cell lines and cell culture

Metastatic colorectal carcinoma cell lines LoVo (target cell) and SW620, non-metastatic colorectal carcinoma cell lines SW480 (control cell) and HT-29, hepatocellular carcinoma cell line HepG2, cervical carcinoma cell line HeLa and lung adenocarcinoma cell line A549 used in the experiment were obtained from American Type Culture Collection. Hepatocellular carcinoma cell lines SMMC-7721 and Bel-7404 used in the experiment were purchased from Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Breast carcinoma cell lines MCF-7 and MDA-MB-231, gastric carcinoma cell lines SGC-7901 and MGC, prostatic carcinoma cell line PC-3M-1E8 were offered by our laboratory. All cell lines used in this study are human derived. The cells were cultured in RPMI 1640 with 10% fetal bovine serum (GIBCO) and 100 U/mL penicillin-streptomycin (Cellgro) at 37 °C in a humidified incubator containing 5 wt%/vol CO_2. Both subculture and pretreatment of cells were completed in the clean bench.



Scheme 1. Schematic representation of metastatic cancer cell and tissue-specific fluorescence imaging using a DNA aptamer developed by Cell-SELEX.

2.2. SELEX library and primers

The library and primers used in the selection were designed by ourselves and synthesized by Sangon Biotech (Shanghai). The library consisted of a randomized region of 40 nucleotides (nt) flanked by two constant regions of 20 nt on both sides for polymerase chain reaction (PCR) amplification (5′-CCTGAACCTGATGCCAACCT-40nt-AGTAGCG AGCGTGTAGTGTG-3′). The forward primer was labeled with FAM (5′-FAM-CCTGAACCTGATGCCAACCT-3′) to monitor the enrichment of the selected pools by using flow cytometry (FACScalibur, BD Bioscience), and the reverse primer was labeled with biotin (5′-Biotin-CACACTACACGCTCGCTACT-3′) to isolate single strand pools from PCR products with streptavidin-coated sepharose beads (GE Healthcare, USA) for subsequent selection rounds.

2.3. SELEX procedures

The initial single strand DNA (ssDNA) library of 5 nmol was dissolved in 100 μL of binding buffer (4.5g/L glucose, 5 mM $MgCl_2$, 1 mg/mL BSA and 0.1 mg/mL yeast tRNA in 0.01 M PBS, pH 7.4). The library was denatured by heating at 95 °C for 10 min and immediately cooled on ice for 10 min. Prior to selection, the cells were treated with non-enzyme cell detach solution (APPLYGEN, Beijing) for 10 min and dispersed in binding buffer. For the first cycle of selection, an amount of 2×10^6 LoVo cells was incubated with the initial library for 1 h on ice. After incubation, cells were washed with 3 mL of washing buffer (4.5g/L glucose, 5 mM $MgCl_2$ in 0.01 M PBS, pH 7.4) three times for 3 min. Cells were centrifuged and transferred to 1000 μL of ultrapure water. The bound ssDNAs were eluted by heating at 95 °C for 10 min. The eluted ssDNAs were amplified by PCR with FAM- and biotin-labeled primers (8–26 cycles of 30 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C), followed by 3 min at 72 °C. The PCR product was incubated with streptavidin-coated sepharose beads for 30 min at 25 °C, then denatured by incubated with NaOH (200 mM) for 10 min. The retaining ssDNA library was desalted and collected for the next round of selection or to monitor the enrichment of the selected library.

Before the 3rd round, the ssDNA pool was incubated only with target cell LoVo (the number of cells was gradually decreased from 2×10^6 to 1×10^6) on ice. After the 3rd round, the pool was first incubated with the negative cell SW480 (the number of cells was gradually increase from 5×10^5 to 5×10^6) to remove nonspecific sequences. The unbound DNA was retained and then incubated with target cell LoVo for positive selection. To improve the specificity and affinity of the selected aptamers, the selection conditions were gradually strengthened by decreasing the positive incubation time from 30 to 60 min and the amount of ssDNA library from 50 to 300 pmol, meanwhile the negative incubation time and the washing time were gradually increased from 30 to 60 min and 1 min to 5 min respectively.

2.4. Enrichment of DNA aptamers

To monitor the enrichment of the selected ssDNA library, 1×10^5 target LoVo cells or control SW480 cells were incubated with 500 nM selected library labeled at the 5′ end with FAM in 200 μL of binding

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