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Diagnostic applications of gastric carcinoma cell aptamers in vitro and in vivo

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ABSTRACT

Gastric carcinoma is the most malignant tumor. Due to lacking of efficient means to diagnose the cancer at the early stage, it is necessary to develop effective molecular probes for early diagnosis and treatment. We have selected aptamers with high specificity and affinity against SGC7901 cells by cell-SELEX (Systematic Evolution of Ligands by Exponential Enrichment) method, which shown important clinical applications: (1) Specific recognize human gastric tumor tissues compared to the normal tissues. (2) When used to capture cancerous cells, the aptamer-functionalized fluorescent-magnetic nanospheres (FMNS) could specifically capture 93% target cancer cells and about70% target cells can be released. (3) The aptamer probe displayed a quenched fluorescence in the absence of target cancer cells and went through a conformational transformation upon binding to target cancer cells that induced fluorescence. (4) The aptamer probe could target gastric tumors transplanted into mice with obvious fluorescence. The newly generated aptamers hold great potential in early cancer diagnosis.

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1. Introduction

Human gastric carcinoma is the most common type of malignant tumors [1], it is important to develop cancer-specific and more sensitive methods.

In recently years, a new type of ligand called aptamer has shown great potential in molecular recognition and targeting [2,3]. Aptamers are evolved through an in vitro selection strategy called cell-SELEX (systematic evolution of ligands by exponential enrichment) from a DNA or RNA library [4–9]. Aptamers are short sequences of nucleotides, such as single-stranded DNA (ssDNA), RNA and modified nucleic acids. These nucleotides can be chemically synthesized and, site-specifically labeled, and therefore site-specifically immobilized. Moreover, because aptamers are much more hydrophilic than antibodies, they may not exhibit nonspecific binding [10].

Due to the rapid development of nanotechnology in science and technology today, its applications has increasingly widespread, the optical properties of nano-materials for the detection of biomolecular have gradually embarked on the stage. The reports are increasing based on magnetic nanoparticles for detection molecules. İsmail Hakkı Boyaci et al. [11] reported the use of antibody coated magnetic nanoparticles for *Escherichia coli* enumeration, this SERS-based sandwich immunoassay possessed a rapid and sensitive feature to target organisms with low detection limit and less detection time. Ramanaviciene et al. [12] reported the use of magnetic gold nanoparticles (MNP-Au) modified by antibodies in oriented or random manner were used for the binding of gp51, enhanced Raman scattering (SERS) was applied as detection method, this immunoassay was successfully applied for the detection of gp51 in milk samples in a rapid, reliable and selective manner.

Since the aptamers have many advantages in used as molecular probes, including low cost of synthesis, high binding affinity and specificity, tunbility and biocompatibility [13–16]. Therefore, aptamers have attracted a great deal of attention for used as biomarkers for cancer diagnosis and treatment. Tan's group developed the

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cell-SELEX strategy to generate a panel of aptamers that can specifically distinguish between any two types of cell lines, more importantly, their newly generated aptamer probes can be used for the molecular profiling of cancer [17–20]. There also have reports on aptamers selected against human gastric tumors [21]. However, until now, the application of aptamers for medical use has been limited due to the scarcity of relevant and practical aptamers. Sreevatan et al. [22] reported the chemical synthesis and functionalization of magnetic and gold-coated magnetic nanoparticles to detect prion protein that was a target to a specific biotinylated aptamer, this study setted the stage for the development of prion detection platforms as well as elucidation the structure at the binding interface.

Because an aptamer selected against cancer cells can specifically bind target proteins of the cancer cells, the aptamer can be designed as a probe to recognize cancer cells in vivo and achieve cancer imaging with high specificity [23]. In the context of molecular imaging, many aptamer probes have been developed [24–26]. However, the same mechanism is not useful when attempting to design an aptamer that can be activated (aptamer-based activas probes) by a certain target [27]. Because aptamer probe sequences can be custom-designed, they can alter their conformation in the presence of the target and in turn transform into signal changes [28]. Based on this concept, the main aim of this study is to develop a series of effective aptamers and apply them to the study of human liver cancer in vitro.

In this report, we used a paired noncancer liver cell line and cancer liver cell line to perform Cell-SELEX. A human gastric cancer cell line, SGC7901cells, as a target and a normal human gastric mucosal cells, GES-1 cell, as a control. We performed the selection process at physiological temperature (37 °C) so that the cells and their membrane receptors were in its biologically active states and generated a panel of new aptamers that can recognize SGC7901cells with high specificity (Kd value in the low nanomolar range) and selectivity (differentiated target cancer cells from other non-targeted cells). We also demonstrated the potential of aptamer in clinical applications. The selected aptamers can specifically recognise human gastric tumor tissues compared to normal tissues. Moreover, when the selected aptamers were used to capture cancerous cells, it showed excellent capture and release efficiency. Based on its secondary structure, we designed an activatable fluorescent aptamer probe to generate a FRET effect in living cancer cells and achieved contrast-enhanced cancer visualization in vitro. In vivo studies demonstrated that activated fluorescence signals were obviously achieved in the SGC7901 tumor sites in mice.

2. Materials and methods

2.1. Cell lines and buffers

SGC7901cell (human gastric adenocarcinoma cells), HeLa cell (human cervical carcinoma cells), A498cell (Human renal cell carcinoma cells), and HT29 cell (Human colon adenocarcinoma cells) were obtained from American Type Culture Collection., and cultured in MEM medium supplemented with 10% FBS GIBCO) and 100 units/ml penicillin–streptomycin (Beyotime), GES-1 cell (Human gastric epithelial cells), MDA-MB-231 cell (Human breast cancer cells) were obtained from American Type Culture Collection, and cultured in DMEM medium contained 10% FBS (GIBCO) and 100 units/ml penicillin–streptomycin (Beyotime). Washing buffer contained 4.5 g/L glucose and 5 mM $MgCl_2$ in Dulbecco's PBS (Hyclone). Binding buffer was prepared by adding 1 mg/mL

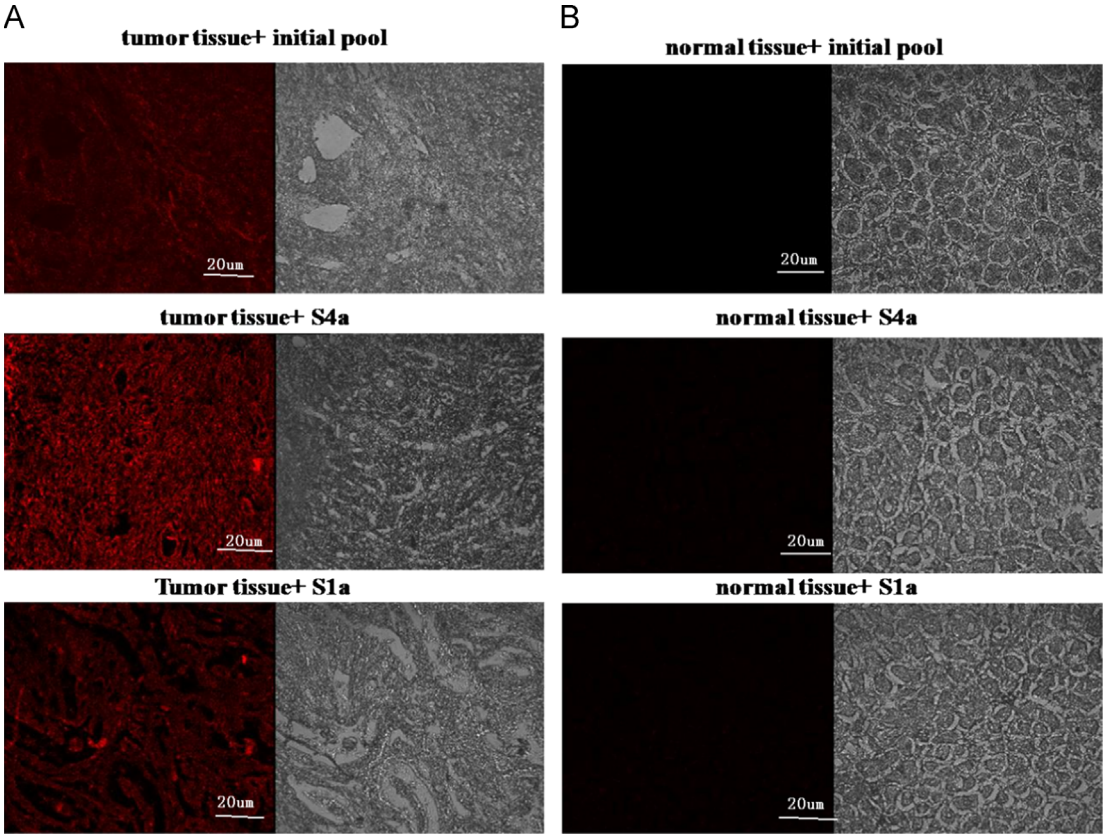

Fig. 1. Confocal images and optical images of formalin-fixed gastric tumor sections (A) and normal gastric sections (B) embedded in paraffin were stained by aptamers S1a and S4a labeled with Cy5 and BHQ2. In each picture, left column is the fluorescent images and right column is the optical images, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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