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Development of a novel imaging agent using peptide-coated gold nanoparticles toward brain glioma stem cell marker CD133

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ABSTRACT

CD133 is known as biomarker for glioblastoma (GBM) and also serves as a marker for cancer stem cells (CSCs), which carry out tumorigenesis and resist conventional therapeutics. The presence of CD133-presenting CSC is a one of the factors in maintenance of the tumorigenic potential of GBM. Thus, CD133 is a potential target for accurate diagnosis of GBM, which could improve its poor prognosis for patients when CSCs are present.

Herein we designed a small peptide-based imaging agent with stimulus-responsive properties. A novel small peptide, CBP4, was screened by a phage display technique, and demonstrated binding to the target CD133 (ECD) comparable to that of an antibody. As a quencher, we used gold nanoparticles (GNPs); the targeting peptide was conjugated to GNPs with high efficiency. By means of a quenching effect, the peptide-coated GNP showed 'signal on–off' properties depending upon the presence of the target. In addition, the particles exhibited biocompatibility when localized in the cytosol. Thus, this study demonstrated that the peptide-coated GNPs can be utilized as an imaging agent for accurate diagnosis of GBM, and further as a drug carrier for therapeutic approaches.

Statement of Significance

The diagnosis and determination of prognosis made by cancer stem cell markers could be a key strategy to eradicate cancer stem cells and cure the cancer. The significance of this study is the characterization of quenching-based signal on–off mechanism and showed that the active targeting via peptide can contribute to the design of a stimulus-responsive cellular imaging agent. Moreover, small peptide based nano complexation showed specific recognition of the target stem cell and internalized on cellular cytosol with stimulus responsive fluorescence. With its novel biocompatibility, the strategy might be a promising tool for drug carrier systems able to measure and visualize the delivered efficiency at intracellular sites.

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1. Introduction

Brain tumors are one of the most feared types of cancer, inducing dysfunctions in the neural circuitry and having poor prognosis when malignant. Glioblastoma (GBM), as the most common type of primary malignant brain tumor, has only a 16- to 18-month median survival rate when multimodal treatment is applied [1]. Despite increasing knowledge of the genetic and molecular characteristics of GBM, therapeutic efficacy has only slowly improved over the past decade [2]. Several studies have shown that the presence of cancer stem cells (CSCs) was one of the factors in the self-renewal of cancer cells to promote tumor growth after conventional GBM therapy [3,4].

CSCs have been identified in various malignant types of cancer, including leukemia and solid tumors [5]. Because they can be self-renewing and pluripotent, CSCs have been indicated as a cause of tumor initiation, metastasis, and drug resistance [6].

CD133 is known as a cell surface antigen allowing identification of CSCs in various types of solid tumors including GBMs [7]. In brain tumors, the existence of CSCs was first assayed using CD133 with their prospective isolation and $CD133^{+}$ CSCs showed stem cell properties including self-renewal and metastasis [8]. Further, it was reported that the CD133 cell surface antigen helps increase the population of tumor initiating cells and has an essential role regarding the maintenance and tumorigenic potential of

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GBM [9,10]. Therefore, CD133 was one of the effective cell surface biomarkers for accurate diagnosis of GBM.

In the present study, we designed a novel target-specific imaging system targeting CD133 as a glioma biomarker. Toward this end, we screened the peptide using the M13 phage library. Phage display has been used to identify a small molecule with target-specific recognition properties using a library of 10^9 random complexity [11]. A random genomic sequence was inserted into the expression vector and cloned using the M13 phage virus to form a phage library at a p3 minor coat protein [12]. Through various screening techniques, the peptide motif as the product possess strong binding affinity to various targets including proteins, hormones, and cells [13]. As a tool for diagnostic systems, peptides have several advantages over antibodies. Peptides, owing to their smaller size (1–3 kDa), show greater penetration of cells and tissues than antibodies [14]. Further, relative to antibodies, peptides have comparable binding affinity, are less immunogenic, less toxic, and simpler to produce [15]. Hence, peptides have various clinical applications and are considered as promising alternatives to antibodies [16].

In the present work, toward the development of a target-specific imaging system based on a small peptide, we prepared citrate-capped gold nanoparticles (GNPs). GNPs have been used widely in visualization and biological imaging systems to identify specific agents, and have several advantages such as biostability, simplicity of synthesis, ease of surface modification and conjugation with biomolecules, and novel optical properties [17,18]. In addition, GNPs have strong fluorescence quenching ability, as much as 100 times that of conventional quenchers [19]. The FRET quenching mechanisms of GNPs have been used as the basis for many prospective biological sensing systems targeting various molecules, from DNA to macromolecules [20,21]. Stimulus-responsive nanocomplexation has been used as a technique to apply GNPs in an imaging system for disease diagnosis [22].

Especially in the tumor microenvironment, various physical and chemical cellular conditions including enzymatic reactions [23], cellular pH [24], and redox reactions [25] and these have attracted interest in the development of stimulus-responsive nanomaterials for antitumor applications. The tripeptide glutathione (L-γ-glutamyl-L-cysteinyl-glycine; GSH) has been identified as an agent that has a cleaving mechanism and exists within tumor cells. GSH is synthesized in cellular cytosol from its precursor amino acids. It has an important role in controlling the cellular cycle and microtubule-related reactions, and acts as a reducing agent in various cellular signaling pathways [26]. In cancer cells, GSH is particularly involved in regulating mutagenesis, DNA synthesis, cell growth, and drug resistance, and also protects the cell against oxidative stress. The drug resistance mechanism of malignant cancer cells is frequently associated with higher levels of GSH than would occur in normal tissue [27]. Nano complexation triggered by GSH has been reported as a promising tool for drug and gene delivery to intracellular targets [28]. This tool relies on the fact that the amount of GSH in intracellular organelles is much higher (5–20 mM) than in extracellular fluids (2–20 μM) [29]; it has also been reported that GSH is of significantly higher concentration in tumor cells than in normal cells [30]. Through the formation of sulfur bonds [31], GSH can enhance the secretion efficiency of delivered and released therapeutic biomolecules within tumor cells [32].

Herein, we designed the novel stimulus responsive imaging tool for detection the brain glioma stem cell. (Fig. 1) To this, we initially identify a novel small peptide that specifically recognizes CD133. This peptide exhibits a specific localization property on cellular cytosol by means of receptor-mediated endocytosis, as observed from confocal microscopy conducted under live cell conditions. Further, the peptide was conjugated with gold nanoparticles as a quenching agent, and the conjugate's stimulus-responsive characteristics were studied in the presence of GSH as the stimulus agent. We finally confirmed the novel fluorescence 'signal on–off' property of the peptide-coated gold nanoparticles as an imaging agent for glioma.

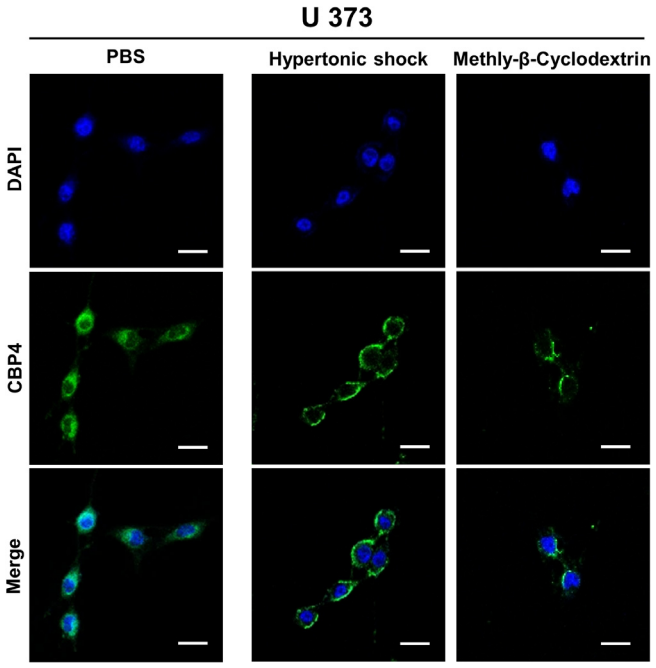

Fig. 1. Endocytic assay using CBP4 on U373 glioma cells. FITC-labeled CBP4 was incubated with U373 human glioma cells for 1 h at 37 °C, followed by laser scanning confocal microscope imaging. Scale bar: 20 μm.

2. Methods

2.1. Immunocytochemistry analysis

U373 glioma cells were used for ICC analysis. The cells were trypsinized with 0.25% trypsin and counted at 0.5×10^5 cells/well. The cells were grown overnight in a tissue culture dish ($35 \times 10\ mm^2$) in 5% CO_2 atmosphere at 37 °C. After incubation, the medium was aspirated and the plate was washed three times using PBS. The cells were pre-incubated with a blocking buffer containing 5% FBS to minimize nonspecific binding. The CBP4, which screened by in vitro M13 phage display(Supporting information), was diluted to 100 nM concentration using a binding buffer (pH 7.2, 50% FBS). In the case of the antibody specific to CD133, the antibody was directly incubated with the cells for 6 h at 4 °C. After incubation, the cells were washed three times using PBS, and nucleus staining was carried out by incubating the cells with 4,6-diamidino-2-phenylindole (DAPI). As a negative control, HEK293T cells were also tested using CBP4 under the same conditions. Finally, the cells were washed three times with PBS and mounted with Dako mounting medium. Stained cells were visualized with a fluorescence microscope (Olympus IX71).

2.2. Sphere cell formation and immunostaining analysis

The U373 glioma cell was subcultured using sphere-forming medium as described previously [33]. A conditioning medium containing epidermal growth factor, (20 mg/mL), fibroblast growth factor (20 mg/mL), and B27, and not containing FBS, was used for sphere formation. For immunostaining of tumor spheres, a 24-well plate was precoated by means of treatment with poly-d-

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