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Molecular recognition of proteolytic activity in metastatic cancer cells using fluorogenic gold nanoprobes

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ABSTRACT

We describe the development of biomarker-sensitive nanoprobes based on nanoparticle surface energy transfer (NSET) effect that enabling recognition of the expression of membrane type-1 matrix metalloproteinase (MT1-MMP) anchored on invasive cancer cells and its proteolytic activity simultaneously. First of all, we confirmed invasiveness of cancer cell lines (HT1080 and MCF7) via migration and invasion assay. We also prepared gold nanoparticle (GNP) acts as a quencher for fluorescein isothiocyanate (FITC). This FITC is conjugated in end-terminal of activatable fluorogenic peptide (ActFP). The ActFP attach to surface of GNP (GNP-ActFP) for a targeting moiety and proteolytic activity ligand toward MT1-MMP. The GNP-ActFP can generate fluorescence signal when ActFP is cleaved by proteolytic activity after targeting toward MT1-MMP. In order to study specificity for MT1-MMP, GNP-ActFP is treated to HT1080 and MCF7 cells, and then, we determine the in vitro targeting potential and fluorogenic activity of GNP-ActFP for MT1-MMP via fluorescence multi-reader. We also confirmed fluorogenic activity of GNP-ActFP via confocal microscopic imaging, and finally, endocytosis of GNP-ActFP is observed via cellular transmission electron microscopic imaging.

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1. Introduction

Generally, many cancers have unique characteristics based on their initiating tissue; abnormal mitoses and chromosomal abnormalities are also observed in cancers (Negrini et al., 2010). For this reason, it is necessary to classify malignant versus healthy tissues in diagnosis and prognosis (Martin et al., 2011; Nowsheen et al., 2012). Despite of the vast efforts in cancer research, cancer continues to be a major cause of mortality. It is therefore important that we develop new methods for the detection and predicting the prognosis of cancer. One actively pursued approach involves seeking various biomarkers of cancers (Nowsheen et al., 2012). A biomarker is a characteristic that can be objectively measured and evaluated in biological samples as an indicator of conditions, such as normal biological processes, pathological states, or pharmacological responses to a therapy, by a variety of techniques (Sawyers, 2008). Biomarkers may then be used to study efficacy and to evaluate safety, detect disease conditions, and monitor health status. Cancer biomarkers have contributed greatly to our understanding of the heterogeneous nature of specific cancers, leading to improvements in treatment outcomes (Georgakilas, 2011). Biological indicator-based diagnostics have

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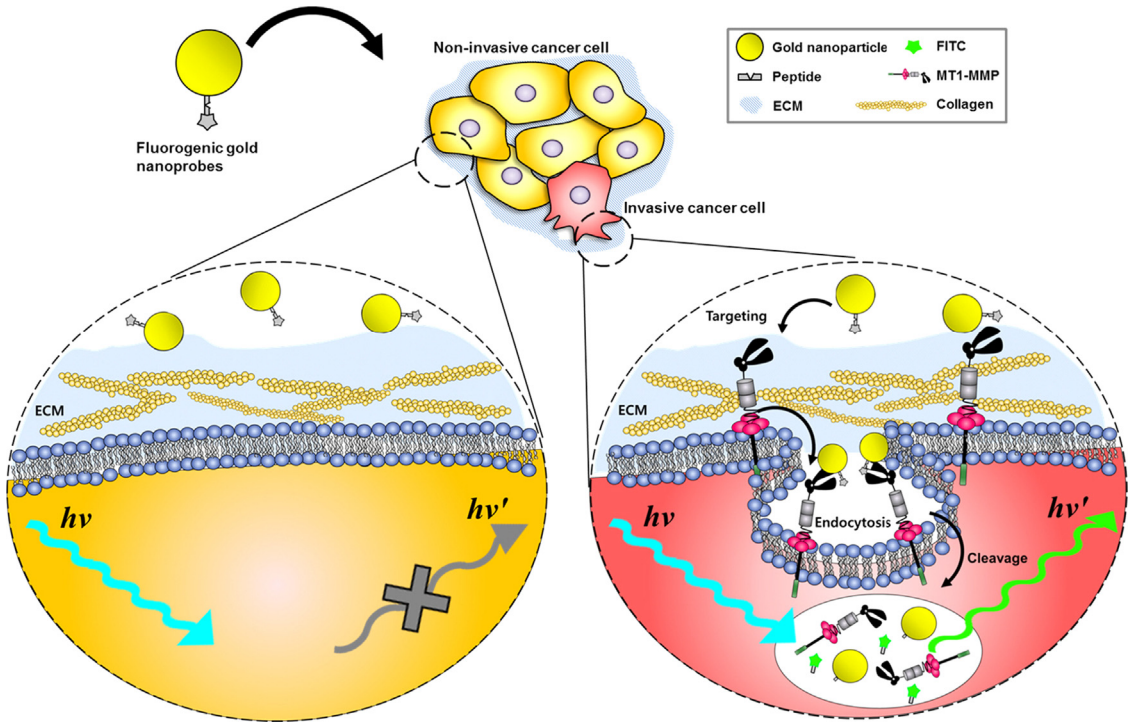

Fig. 1. Schematic illustration of fluorogenic nanoprobes (gold nanoparticles conjugated with activatable fluorogenic peptide, GNP-ActFP) for detection of MT1-MMP anchored on invasive cancer cells using fluorescence signal and imaging.

applications for establishing disease predisposition, early detection, cancer staging, therapy selection, identifying whether a cancer is metastatic, therapy monitoring, assessing prognosis, and advances in the adjuvant setting (Ferrari, 2005; Lopez-Otin and Matrisian, 2007).

Among cancer biomarkers, proteinases have long been associated with cancer progression, because of their ability to degrade extracellular matrices. Proteinases have also been extensively studied because of their regulatory roles in many metabolic processes (Edwards and Murphy, 1998; Lee et al., 2009b). Among the proteinases, matrix metalloproteinases (MMPs) are known to not only be important in normal tissue remodeling, but to also play important roles in many diseases, such as atherosclerosis and cancer (Overall and Kleifeld, 2006; Zheng et al., 2007). In the large family of MMPs, cell membrane-anchored proteinases and membrane-type (MT) MMPs especially draw attention because of their fundamental roles in cancer cell metastasis and proliferation (Deryugina et al., 2005; Zhai et al., 2005). In particular, MT1-MMP provides a direct cellular target for molecular imaging to detect invasive cancer cells as well as playing important and essential roles (Hotary et al., 2003; Park et al., 2012).

Inorganic nanoparticles (NPs), including gold NPs (GNPs), magnetic NPs, and quantum dots, have been investigated extensively for nucleic acid-detecting sensors, molecular imaging probes, and protein purification processes (Lee et al., 2006; Oh et al., 2006a). Among the inorganic NPs, GNPs are of great interest because of their effective fluorescence-quenching ability when fluorescent molecules are located in their vicinity. This quenching behavior, based on NP surface energy transfer (NSET), gives an outstanding low signal-to-noise ratio and provides simultaneous multiple fluorescence quenching effects at differing energies (Guarise et al., 2006; Lee et al., 2009a; Oh et al., 2006b).

In a previous study, we developed a bimodal imaging nanoprobe using a magnetic nanocrystal conjugated with a fluorogenic peptide as a magnetic resonance imaging agent and an infrared imaging agent simultaneously (Park et al., 2012). However, this study only focused on the coincident imaging ability of the nanoprobe and there was no effort to improve the sensitivity of its MT1-MMP detection level. Additionally, we conducted real-time quantitative in vitro assays using atomic force microscopy for MT1-MMP detection, but the detecting process was delicate and time-consuming (Lee et al., 2012).

Here, we describe the development of a nanoprobe for precise recognition of MT1-MMP expression levels anchored in invasive live cancer cells and its proteolytic activity (Fig. 1). For specific targeting towards MT1-MMP anchored on the cell surface, we used an activatable fluorogenic peptide (ActFP) that was designed and synthesized as both a MT1-MMP-specific substrate and a fluorescence-imaging probe, based on the NSET effect (Fig. S1).

2. Materials and methods

2.1. Characterization of cancer cell

Migration and invasion kinetics of HT1080 and MCF7 cells were analyzed using the xCELLigence DP system (Roche Diagnostics GmbH, Germany) (Limame et al., 2012). For monitoring of migration, cancer cells (HT1080 and MCF7 cells) were seeded in the upper chamber in serum-free medium at a density of 4×10^4 cells/well. The upper chamber was then placed on the lower part of the CIM-Plate 16 Assembly Tool (Roche Diagnostics GmbH) containing growth medium supplemented with 10% fetal bovine serum (FBS) to promote migration across the membrane towards the serum gradient. Migration of cancer cells was followed for 24 h by tracking changes in the impedance signal in a CIM plate, measured on the opposing side of the membrane.

For the invasion assay, protocols identical to those for migration were followed, except that the upper chamber were loaded with 20 μL of a 1:20 dilution of Matrigel (BD Biosciences, USA) to create a 3D biomatrix film in each well prior to cell loading. Each experiment was performed in triplicates and repeated twice. For

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