Supporting Information

A DNA-directed covalent conjugation fluorescence probe for *in vitro* detection of functional matrix metalloproteinases

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1. Principle of Ultra-4 centrifugal filters

The removal of excess SPDP and maleimide-NTA was operated by ultra centrifugal filters (3 KD), which means molecule weight smaller than 3 KD being removed and larger being kept. The removal of small molecule such as SPDP and maleimide-NTA can be accomplished in the Amicon Ultra-4 device by concentrating the sample, then add solvent such as water and PBS to original sample volume. The process of "washing out" can be repeated until the concentration of the contaminating microsolute has been sufficiently reduced. See example below. The amount of microsolute was regarded as a mg, after 3 times centrifugation, the amount of microsolute was remained about a/512000 mg, which can be neglected.

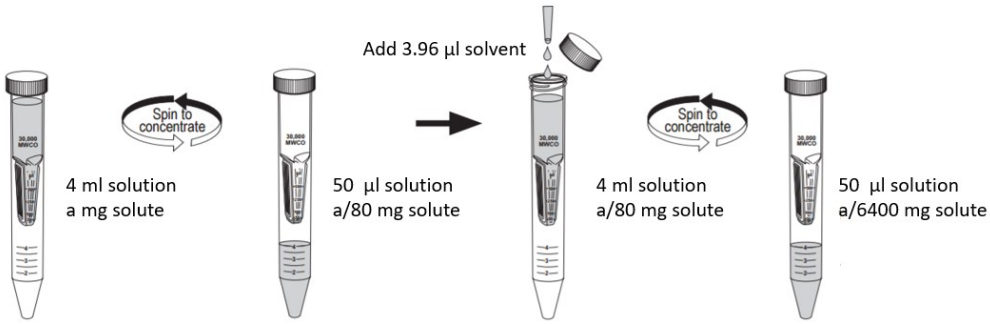

Fig. S1 Principle of Ultra-4 centrifugal filters to remove the excess agent.

2. MALDI-MS analysis

MALDI-MS analysis was performed in an AXIMA Performance MALDITOF/TOF mass spectrometer (Shimadzu Co. Ltd., Japan). This instrument was equipped with a 337 nm nitrogen laser.3-Hydroxypicolinic Acid (3-HPA, Sigma Aldrich, USA) was chosen as MALDI matrix for DNA detection. The matrix solution was prepared by dissolving 20 mg 3- HPA and 45 mg dihydrogen ammonium citrate (DHAC) in 1 mL mixture solution of 50% acetonitrile/50% water. Data were acquired in a linear negative mode and signals between m/z 3000–7000 were collected. The scan area was 200 µm × 200 µm with the sampling distance of 50 µm. For each coordinate, mass

spectra resulting from 20 laser shots at 5 Hz were accumulated to obtain an average mass spectrum.

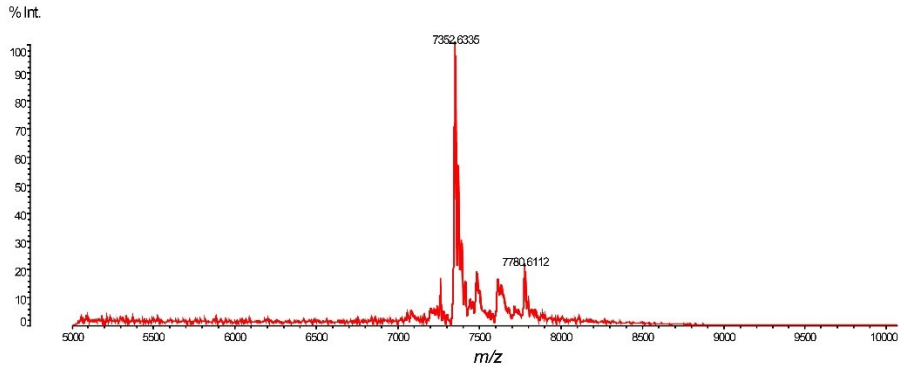

Fig. S2 Mass spectra of DNA-directed NTA-modified probe. The peak of 7352.6335 represents intermediate product containing thiol group. And the peak of 7780.6112 refers to the probe we prepared.

3. Evaluation of affinity between aptamers and MMP-9

To evaluate biolayer interferometry for studies of the combination between two kinds of aptamer and MMP-9, we load Amine Reactive 2nd Generation (AR2G) biosensors to provide a surface with a high density of carboxylic acids, move the sensors to a reaction buffer containing EDC (1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride) and s-NHS (N-hydroxysulfosuccinimide) to generate highly reactive NHS esters. The esters rapidly react with the primary amines of RNA aptamer to form highly stable amide bonds. The response reached with the blocking step serves as a baseline for subsequent steps. Next, sensors are placed into solutions of MMP-9 to evaluate the association step, and subsequently placed back into the blocking/baseline solution. Finally, second circle-template aptamer was used to combine with sensors for the determination of affinity properties. The data collected are processed and analyzed with the Octet Data Analysis Software.

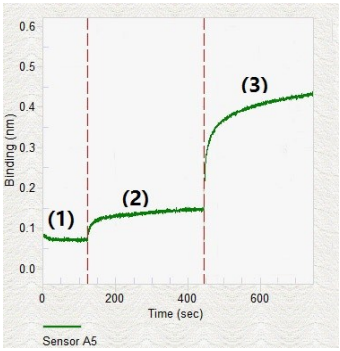

Fig. S3 Evaluation of affinity between aptamers and MMP-9. (1) After activation, covalent immobilization of RNA aptamer on AR2G biosensors. (2) Affinity association of MMP-9 with the sensor (3) The combination of DNA aptamer with sensor. Each step has a good combination according to the height of binding in the vertical coordinates.

4. Immunofluorescent analysis of glioma stem cells

Cells were seeded on a Confocal dish and grown for 24 h before the experiment. Cultures were then fixed with 4% paraformaldehyde, permeabilized with 0.2% Triton，and blocked with 5% BSA. Cells were incubated in primary antibody of CD133 antibody (host: rabbit) and nestin antibody (host: mouse) at a concentration of 1:100 and 1:500 in primary antibody diluent, respectively. Then, cells were incubated in secondary antibody of FITC goat anti-rabbit

and Cy3.5 goat anti-mouse IgG. Subsequently, these cells were incubated with a anti-fade 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) solution (1:1000; Dojindo Laboratories, Inc., Kumamoto, Japan) for nuclear staining. All microscopy measurements were performed on Zeiss780 inverted fluorescence microscope.

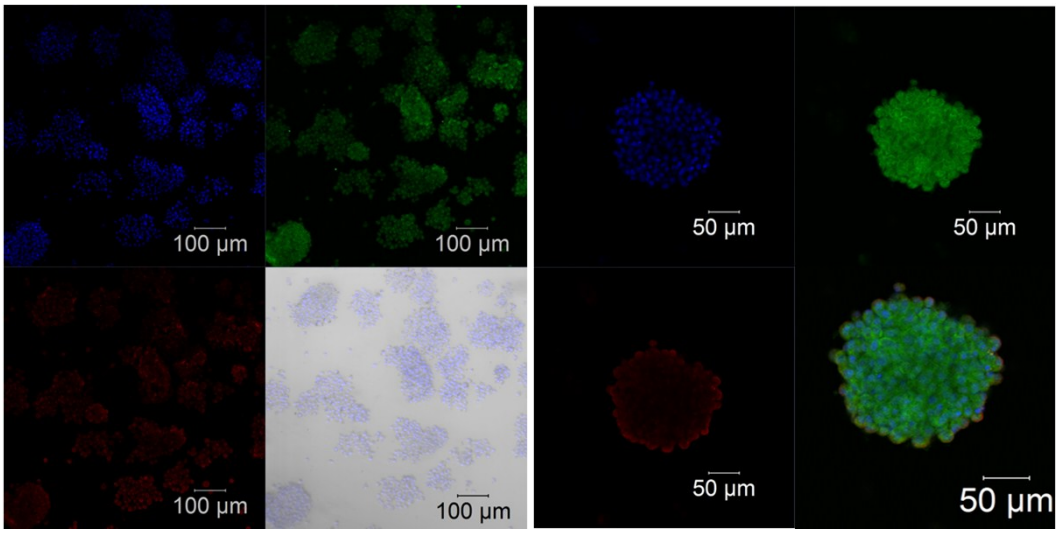

Fig. 4 Imaging results of immunofluorescent analysis of glioma stem cells. (a) Fluorescence imaging result of cell nucleus(blue) in GSC cells in 10×ocular. (b) Fluorescence imaging result of CD133(green) in GSC cells in 10×ocular. (c) Fluorescence imaging result of nestin(red) in GSC cells in 10×ocular. (d) Bright field imaging result of GSC cells in 10×ocular. (e) Fluorescence imaging result of cell nucleus(blue) in GSC cells in 40×oil ocular. (f) Fluorescence imaging result of CD133(green) in GSC cells in 40×oil ocular. (g) Fluorescence imaging result of nestin(red) in GSC cells in 40× oil ocular. (h) 3D merged fluorescence image of GSC cells.

Table 1.

The preparation of different concentration of three kinds of proteases

Superoxide Dismutase						
Number	1	2	3	4	5	6
Stock solution /μl (5KU/ml)	0	1.5	3	4.5	6	7.5
H2O/ul	10	8.5	7.0	5.5	4	2.5
Concentration/ (U/ml)	0	750	1500	2250	3000	3750

Transferrin						
Number	1	2	3	4	5	6
Stock solution/μl (2.5mg/ml)	0	2	4	6	8	10
H2O/ul	10	8	6	4	2	0
Concentration/ (mg/ml)	0	0.5	1.0	1.5	2.0	2.5

MMP-9						
Number	1	2	3	4	5	6
Concentration/ (ng/ml)	0	31.25	62.5	125	250	500