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Paper

Peptide-based magnetic nanoparticles for isolation of circulating tumor cells

Supplementary Information

Linling Bai, [a,d] Yimeng Du,[a] Jiaxi Peng,[a,b] Yi Liu,[c] Yanmei Wang,[a] Yanlian Yang*[a] and Chen Wang*[a]

1. Flow cytometry

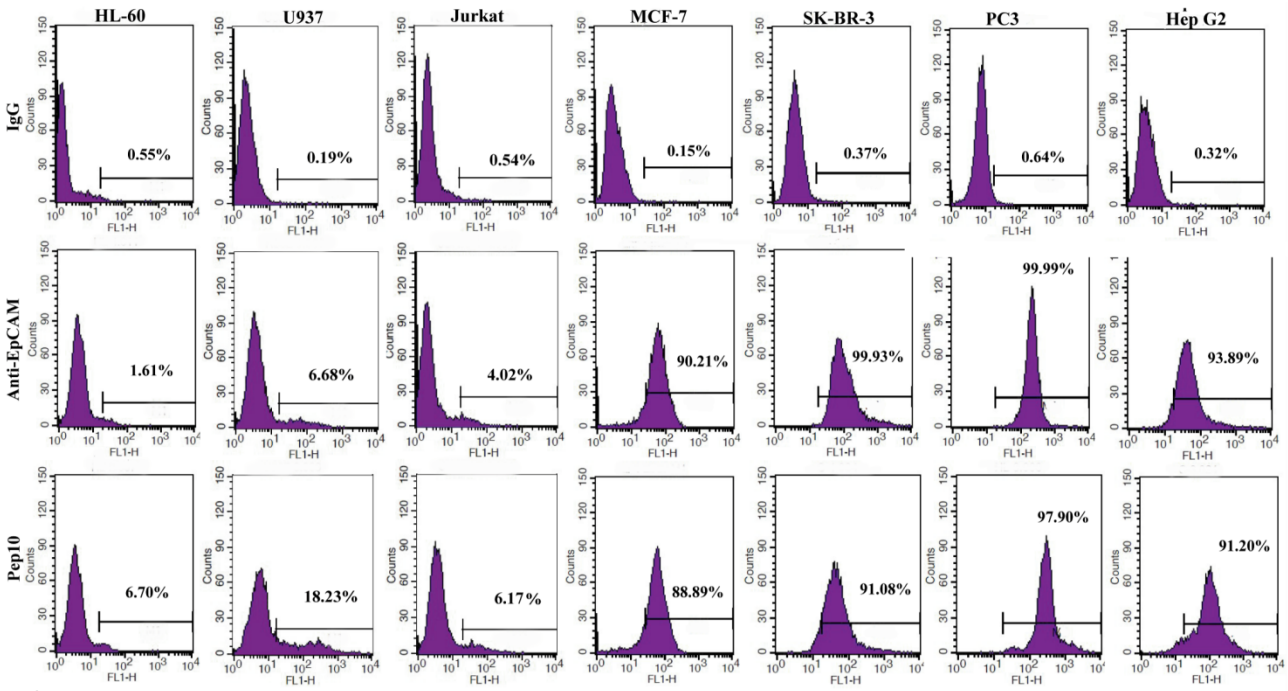

Fig.S1 Detection of FITC-labeled Pep10 bound to HL-60, U937, Jurkat (EpCAM negative cell lines) and MCF-7, SK-BR-3, PC3,Hep G2(EpCAM positive cell lines) cell lines. FITC-labeled anti-EpCAM and IgG2bκ isotyre controls are shown for comparison.

The binding abilities of the FITC-labeled Pep10 in EpCAM positive cell lines: MCF-7, SK-BR-3, PC3, Hep G2 as well as EpCAM negative cell lines: HL-60, U937 and Jurkat were investigated by FCM, respectively. FITC-labeled anti-EpCAM and IgG2bκ were chosen as the controls. The typical results representative of three repeated experiments were shown in the Fig.S1.

2. SPR

Figure S2 shows the SPR sensorgrams of peptides binding with the recombinant human EpCAM protein. Association and dissociation took place while the EpCAM flowed through the gold chip modified with Pep10.

$$A+L \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AL \quad (1)$$

where A is the analyte (recombinant human EpCAM), L is the immobilized ligand (Pep10), and AL is the complex of A binding to L.

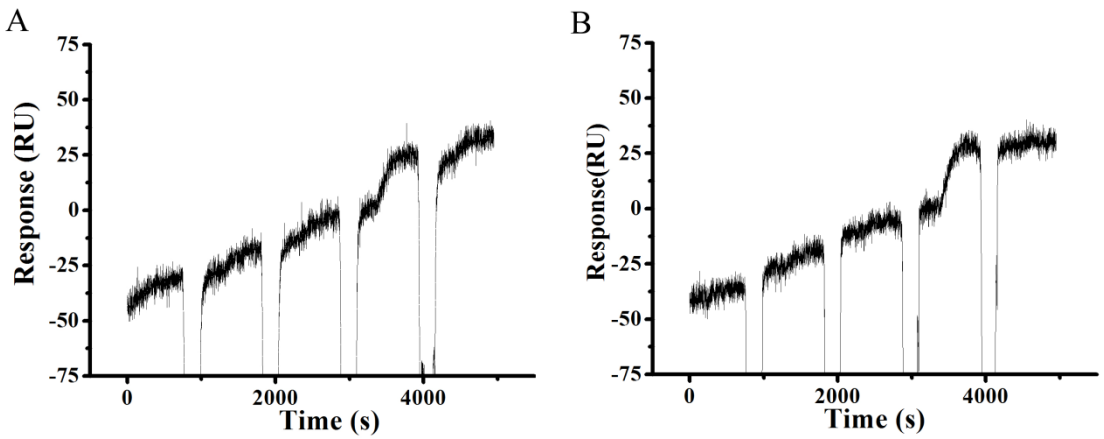

Fig.S2. SPR response on biosensor of (A) anti-EpCAM (B) Pep10 reference to EpCAM. The graph contains five steps, where each step contains one uphill association curve and one downhill dissociation curve. The five steps correspond to five different concentrations of EpCAM, 1.56, 3.12, 6.25, 12.50, 25.00 μg/mL.

The association rate equals to $k_aC(R_{max} - R)$, and the dissociation rate equals to k_dR, where k_a is the association rate constant, k_d is the dissociation rate constant, C is the concentration of EpCAM, R_{max} reflects the maximum amount of EpCAM that can be captured on the peptides surface, which theoretically means the maximum response that can be achieved when the concentration of EpCAM passing through the peptide surface and contact time are infinite, and R is the response to the binding of EpCAM. At equilibrium, the association rate equals to the dissociation rate; therefore

$$k_aC(R_{max} - R_{eq}))= k_dR_{eq} \quad (2)$$

that is

$$\frac{1}{R_{eq}} = \frac{1}{R_{max}} + \frac{1}{K_A} \frac{1}{R_{max}} \frac{1}{C} \quad (3)$$

here, the affinity constant, K_A equals to k_a/k_d. The plot of $1/R_{eq}$ against $1/C$ is a straight line with the value of slope being $1/(K_AR_{max})$ and the intercept $1/R_{max}$. Consequently, the value of affinity constant K_A can be obtained from the slope and intercept. Moreover, K_D equals to $1/K_A$. The value of K_D for Pep10 binding with EpCAM was determined to be 1.98×10^{-9} mol · L^{-1} by SPR technique.

For the SPR analysis, we have developed a non-regeneration kinetic method in which there was no regeneration between consecutive injections. Using the non-regeneration protocol, R_{eq} for each injection could be obtained, which was plotted in Fig.S2 accordingly. For

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binding of EpCAM and anti-EpCAM, our SPR study was in close agreement with the results reported by Shan X. Wang *et al* [1-2].

3. Quantification of peptides conjugated to MNP surfaces

To measure the amount of peptide loaded onto the surface of MNPs, a series of FITC-Pep10-biotin solutions (50, 100, 200, 400, and 500 μg/mL) in PBS buffer were prepared and used to create a standard curve for the UV/Vis measurements. Then the optical density (O.D.) at 495 nm (OD_{495nm}) of peptides solution was obtained with plate reader (TECANInfinite-M200, Männedorf, Switzerland). The OD495nm values were plotted versus the peptide concentrations as a standard curve (Fig. S3). The linear fitting of the standard plot will give the following equation:

$$Y=A+B*X \quad (4)$$

where Y is the actual OD_{495nm} of peptide, constants A and B are both obtained from linear fitting, and X is the amount of the peptides in solution in μg/ml.

After functionalization of the peptides onto MNPs, the MNPs are centrifuged and the OD_{495nm} of the supernatant solution was obtained. From the standard curve, the corresponding amount of peptide in the solution can be easily calculated, which is the unbound peptides. Then, the amount of peptide on MNPs surface can be calculated.

$$\text{Amount of peptide on MNPs surface} = \text{Amount of peptide added} - \text{Amount of peptide unbound} \quad (5)$$

After calculation, 200 nm MNPs functionalized with Pep10 showed the conjugation efficiency of about 20.3 nmol/mg (Fe).

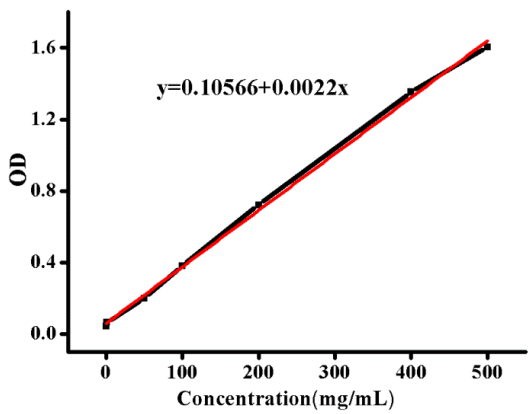

Fig.S3. The standard curve of OD_{495nm} versus the concentration of FITC-Pep10-biotin.The black line was the experimental data obtained at concentrations of 50, 100, 200, 400, 500 μg/mL. The red line was the line fitting curve using software origin8 (R-Square is 0.999819).

4. Isolation of cancer cells with peptide-conjugated MNPs

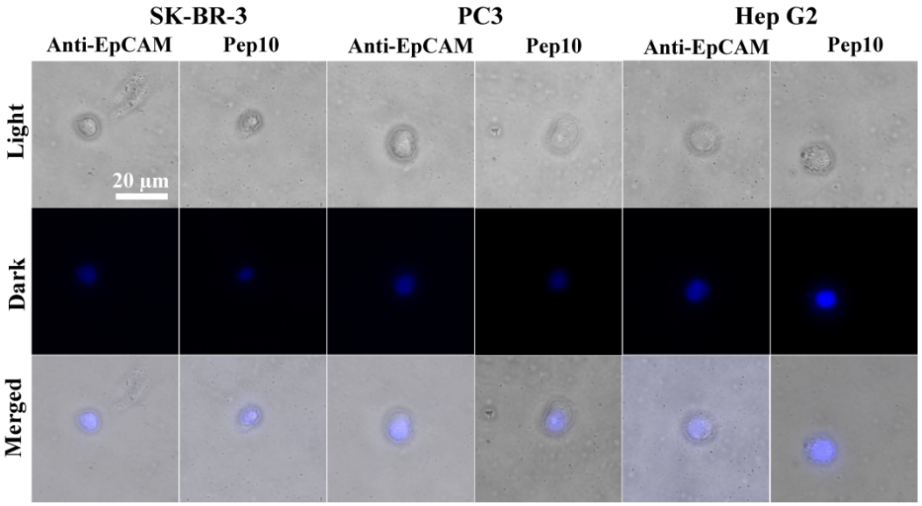

Fig.S4. Fluorescence microscopic images of captured cells in mixture of SK-BR-3, PC3, Hep G2 cell and human whole blood at the concentration of 50 cells·mL^{-1} by Pep10@MNPs and anti-EpCAM@MNPs.

To demonstrate the effectiveness of Pep10@MNPs to capture CTCs in whole blood, SK-BR-3, PC3, Hep G2 cells respectively spiked in fresh human blood were used in the present study. During the cancer cell separation process, the 50 Hoechst.33342 pre-stained cancer cells were added to 1 mL human blood. The spiked blood was exposed to the Pep10@MNPs to allow for the capture of cancers cells by Pep10@MNPs. As shown in Fig. S4, the cancer cells were successfully captured directly from spiked fresh whole blood with Pep10@MNPs.

5. Cytotoxicity assay

The *in vitro* cytotoxicities of Pep10 were determined with MCF-7, SK-BR-3, PC3 and Hep G2 cells by MTT assay. Briefly, the cells were seeded in 96-well plates at a density of 4000 cells per well, and then incubated in 80 μL RPMI-1640 containing 10% FBS for 12 hours. After that, 20 μL Pep10 solutions with concentrations of 0.5, 5.0, 50.0, 500.0 and 1000.0 μg/mL were added separately. After 48 hours of incubation, 15 μL MTT (5 mg· mL^{-1} in PBS buffer) solutions were added to each well and further incubated for 4 h. After that, 100 μL sodium dodecyl sulfate (SDS) solution were added in each well. The absorbance was measured at 570 nm using a microplate reader (Tecan infiniteM200，TECAN，Swiss Confederation). The relative cell viability was calculated as:

$$\text{Cell viability (\%)} = (OD_{570}(\text{samples})/OD_{570}(\text{control}))\times 100\% \ . \quad (6)$$

Each value was averaged from three independent experiments.

For understanding the influence of the peptides on the tumor cells, the cytotoxicity of Pep10 to MCF-7, SK-BR-3, PC3 and Hep G2 cells were investigated by MTT assay. As shown in Fig. S5, average cell viability of MCF-7, SK-BR-3, PC3 and Hep G2 was above 80% after 2 days culturing with Pep10 at the concentration of 200 μg·mL^{-1}, which indicates that Pep10 didn't show pronounced cytotoxic effects on tumor cells. So, the recognized peptide Pep10 could be used for CTC capture safely.

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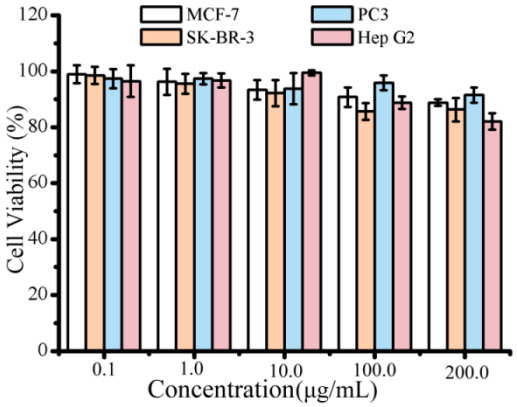

Fig.S5 Cell viability of MCF-7, SK-BR-3, PC3 and Hep G2 at added Pep10 concentration.

Notes and references

[a] *CAS Key Laboratory of Standardization and Measurement for Nanotechnology, National Center for Nanoscience and Technology, Beijing 100190, P.R. China. Fax: 86-10-62656765; Tel: 86-10-8254555; Email: yangyl@nanoctr.cn;*

Fax: 86-10-62656765; Tel: 86-10-82545561; Email: wangch@nanoctr.cn

[b] *Department of Chemistry, Renmin University of China, Beijing 100872, P.R. China.*

[c] *Translational Medicine Center, Laboratory of Oncology, Affiliated Hospital of Academy of Military Medical Sciences，Beijing 100071, P.R. China.*

[d] *Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, P.R. China.*

† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

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